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MORTALITY OF THE RED SCALE ON CITRUS THROUGH INFECTION WITH A SPORE-FORMING BACTERIUM

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(Accepted for publication June 26, 1941)

Bacterial parasites of the California citrus red scale, *Aonidiella aurantii* (Mask.), have, so far as we know, never before been isolated or studied. It appeared of interest, therefore, to devote some attention to a microorganism recently proved to be a pathogen of red scale on citrus and an occasional inhabitant of dead citrus scale in some orchards. This organism was first isolated from soil in connection with denitrification studies and grown on chitin, cellulose, and a large number of organic media. It is tentatively designated Bacillus "C."

Under certain laboratory conditions it has been found possible to bring about a wholesale invasion and death of the red scale by Bacillus "C," especially on lemons.

THE MICROORGANISM

Bacillus "C" is a large (6 by $1\frac{1}{4}$ μ) Gram-positive motile rod, which forms spores in the equatorial position. Its motility is contingent upon the presence of clumps of several polar flagella and is lost, as a rule, after a few days of growth on the ordinary laboratory media. The microorganism grows singly, in couples joined end to end, and in chains of 4 or more (Fig. 1).

Bacillus "C" is an aerobe. It can grow throughout a number of liquid media in the presence of either nitrate or nitrite, provided oxygen is not entirely excluded, as in cotton-stoppered flasks or test tubes. On Spray anaerobic plates, Bacillus "C" develops no growth, even in the presence of nitrate. Ammonia and a still unidentified nitrogenous substance frequently appear to be the products of the reduction of nitrate or nitrite. Within a rather narrow optimum, on the other hand, nitrates appear to be ultimately reduced to the gaseous forms of nitrogen.

On aerobic plates, Bacillus "C" grows within the entire pH range studied, that is, from pH 4 to pH 9.5. In liquid cultures an optimum in the vicinity of pH 7.5 is suggested, while growth is limited to the active acidity range of pH 6 to pH 9.3. The optimum temperature for the growth of Bacillus "C" in the suspensions of chitin is in the vicinity of 30° C. It is able to grow, however, at 12° and at 40° C.

Bacillus "C" was originally isolated from the following enrichment medium:

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Filter paper, Whatman No. 42, dispersed in the Waring blender	10 per cent
Potassium nitrate, Baker's	1 " "
Potassium hydrogen phosphate, Baker's	1 " "
Magnesium sulphate heptahydrate, Baker's	½ " "
Calcium carbonate, Mallinkrodt's	2 " "

Redistilled water (all-glass still)

Inoculum: 5 grams of surface soil from the Citrus Experiment Station.

The original enrichment culture was incubated in a 50-cc. glass-stoppered Pyrex bottle at 28° C. for about 1 week. Transfers were made into wide-mouthed 500-cc. Pyrex Erlenmeyer flasks stoppered with cotton, containing 300 cc. of sterile medium of the same composition as the enrichment culture (Fig. 1, A). After 2 successive transfers, the mixed culture was plated on 2 per cent agar, containing the same filter paper and salts as the original enrichment medium. At the same time platings of the mixed culture were made on peptone-yeast agar. Among the organisms developed on these plates were two bacteria that grew equally well on filter paper, peptone-yeast, and chitin agar. One of these, a spore-former, was chosen for further observation.

In general outline and in manner of sporulation there appear to be certain resemblances between Bacillus "C" and the microorganism described by Dutky (3).

EXPERIMENTAL PROCEDURE

Field lemons, infested with the red scale, were used almost exclusively in the investigations here reported. These lemons were collected in 2 different orchards: one in the vicinity of Corona, California; the other in the City of Riverside. Lemons from the latter grove had not been fumigated nor otherwise treated for the red scale during the preceding 10-year period. Hand-picked lemons were brought into the laboratory and carefully washed with soap. It was found necessary also to wash them for 1 or 2 minutes in 0.1 per cent copper sulphate solution to minimize the importance of fungi as an interfering factor. Neither soap nor copper sulphate, however, was found to remove or destroy all of the indigenous bacteria;[3] hence it cannot be stated that the work presented here was done under sterile conditions.

Bacillus "C" was conditioned for the infection in the following manner: 48 hours before the experiment, 500-cc. aliquots of peptone water,[4] in a 1-liter Pyrex Erlenmeyer flask stoppered with cotton, were inoculated with a loopful of bacteria from a pure-culture slant. When suspensions of the

[3] One of these indigenous bacteria in particular appeared to be interesting on account of its ability to grow rapidly on chitin, and to produce a characteristic dark-yellow pigment under a variety of conditions. It was possible several times to isolate this pigment producer from crushed bodies of the insects that were already dead and dried on the fruit. It did not prove, however, to be pathogenic to the normal scale under the conditions of the present study.

[4] Composition of the peptone water:

Proteose peptone, "Difco"	1.0 per cent
Potassium nitrate, Baker's	0.1 " "
Potassium hydrogen phosphate, Mallinkrodt's	0.1 " "
Magnesium sulphate heptahydrate, Baker's	0.05 " "

Tap water, pH 7.5–7.6 (glass electrode)

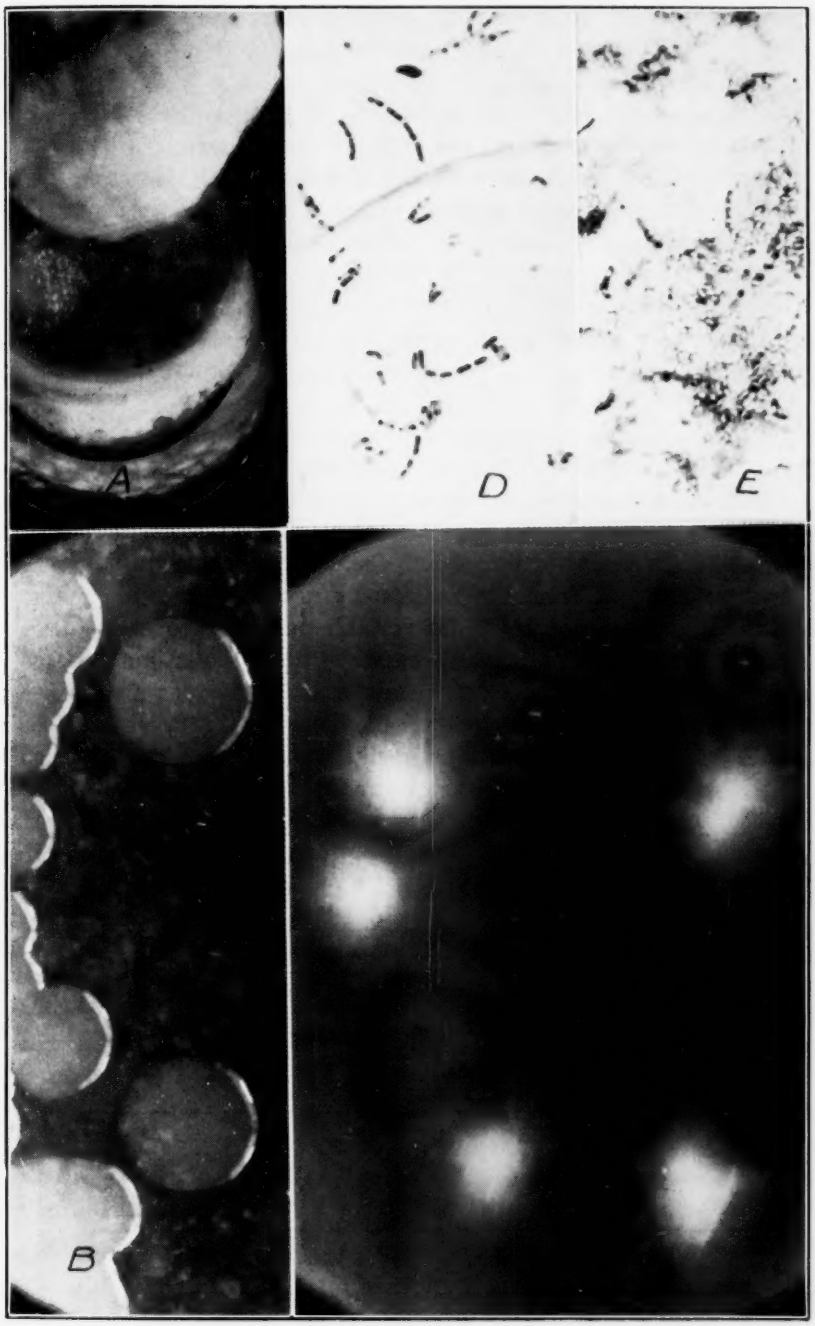

FIG. 1. Bacillus "C." A. Active transfer from the filter paper nitrate-enrichment medium. B. Two-day colonies on peptone agar. ×12. C. Two-day colonies on citrate-asparagin agar. ×12. D. From peptone-nitrate broth (Gram stain). ×1,500. E. Unstained spores from chitin agar. ×1,500.

bacillus in tap water or in buffered distilled water were used, 48-hour-old aerobic growth on peptone-agar plates was scraped off and suspended, under sterile conditions, in 500-cc. aliquots of water.

It was found desirable to maintain both the fruit and the bacterial cultures at the same temperatures (28° C.) for approximately 6 hours before the experiment. Single fruits were then placed for variable periods of time in beakers containing either peptone-water cultures or suspensions of the bacillus in sterile tap water.

Parallel "treated controls," in which sterile peptone broth or sterile tap water only was used, were provided for all tests in all cases. "Untreated controls" were likewise maintained. These were fruits incubated alongside infected and "treated control" fruits, without any treatment whatsoever.

It was found that in order to obtain a satisfactory mass infection of the scale, 2 to 4 hours' immersion was necessary. In the majority of cases, however, a 16-hour immersion was preferred because of the consistency and the reliability of the results ensured by the longer wetting period. At the expiration of the period of immersion, the controls, as well as the infected fruits, were removed from the beakers by means of crucible tongs and placed in the incubator at 28° C. for variable periods of time. Under these conditions, it made no difference whether in order to obtain satisfactory results the fruit was kept in a moist chamber or in the open air. When the fruits were immersed for shorter periods, however, the moist chamber appeared to be indispensable. At the conclusion of the incubation period, all adult female insects were removed from the fruits and examined under the microscope, individually, for general appearance, movement of the pygidia, and a few tentatively defined symptoms of the infection (Table 1),

TABLE 1.—*Visible signs of infection of citrus with Bacillus "C"*

Insects	Total number examined	Adult insects: Per cent showing: Browning	Adult insects: Per cent showing: Distortion of pygidia	Adult insects: Per cent showing: Disintegration	Crawlers: Total number examined	Crawlers: Per cent mortality
Infected with Bacillus "C"	1120	66	56	32	965	98
Controls (treated)[a]	575	47	56	22	191	57
Controls (untreated)	602	18	28	11	353	25

[a] Treated in the same manner as the infected ones except for the absence of Bacillus "C."

When chemical analyses of normal and of infected adult insects were undertaken (in lots of 100), every insect in the lot was examined under the microscope prior to the analysis in order to ensure, so far as possible, complete uniformity in the condition of the insects. Aqueous extracts of the

insects chosen for the analysis were made in the following manner: One hundred adults were crushed with about 5 cc. of distilled water and 1/10 g. of copper sulphate. About 50 cc. of distilled water was then added, and the extract was boiled in an Erlenmeyer flask under a reflux condenser for 2 min. Approximately 1/10 g. of the secondary potassium phosphate was added after the digestion; also 1/10 g. of calcium carbonate and a trace of magnesium oxide. One g. of "Norit" activated carbon was finally introduced, and the mixture was brought rapidly to the boiling point. The hot extract was filtered through Whatman No. 42 filter paper. The residue on the filter was washed with about 100 cc. of boiling distilled water. The combined filtrate and washings were cooled and made up to 250 cc. with distilled water. Aliquots of this colorless transparent filtrate were taken for the colorimetric determinations of nitrate and nitrite. Nitrite was determined by the Griess reagent, following the usage of Elema (5). Nitrate was determined by the official phenoldisulphonic-acid method (1).

Total nitrogen was determined on separate lots of the insects by means of the Gunning-Hibbard modification of the Kjeldahl procedure (1). The acid digestion was made in micro-Kjeldahl flasks. For the distillation, the ordinary Kjeldahl apparatus was used.

THE INFECTION

Examinations of the infected adult red scales often show Bacillus "C" adhering to the waxy membrane of the lower margin of the insect, especially in the proximity of the pygidial cavity. When insects, detached from the fruit, are wetted with suspensions of the bacillus, it is sometimes possible to observe the latter penetrating the pygidial cavity and apparently entering the insect through the oviduct.

Crawlers are attacked by the bacillus with far greater rapidity than the adult insects. Clumps of the bacilli are frequently formed in the vicinity of the joints of the crawler's legs, and in less than 15 minutes after the formation of a well-defined clump, the crawler succumbs. In the case of the adults, on the other hand, pygidial movements cease only after approximately 25 minutes of the contact with the bacillus.

After but a few days of incubation in the dry at 28° C., a large proportion of the infected adult insects exhibit a rather characteristic browning of the ventral surface. This browning is sometimes limited to the areas adjoining the pygidial cavity or to the vicinity of the mouth. Brown spots may be observed occasionally on the prosomatic lobes and on the surface of unhatched embryos or eggs inside the insect.[5] An expulsion of dead crawlers and embryos, especially on grapefruit, takes place occasionally

[5] Similar browning of the ventral surface of the insect also was found to be a consequence of 1 hour's immersion in 0.1 M solution of hydroxyl amine containing pH 7.5 phosphate buffer. Comparable treatments with nitrites, nitrates, and ammonium salts caused no browning. Neither was it observed in the insects treated with hydrogen sulphide or hydrogen cyanide. Natural illuminating gas, however, and 5 days' storage over pyrogallol in sealed desiccators were found to produce browning that resembled, superficially, the browning caused by the bacillus.

during this stage of the infection. There is a general darkening in the color of the infected insects that is visible to the naked eye (Fig. 3). Pygidia, viewed under the microscope, sometimes appear distorted, that is, twisted, collapsed, irregularly swollen, or otherwise deformed.

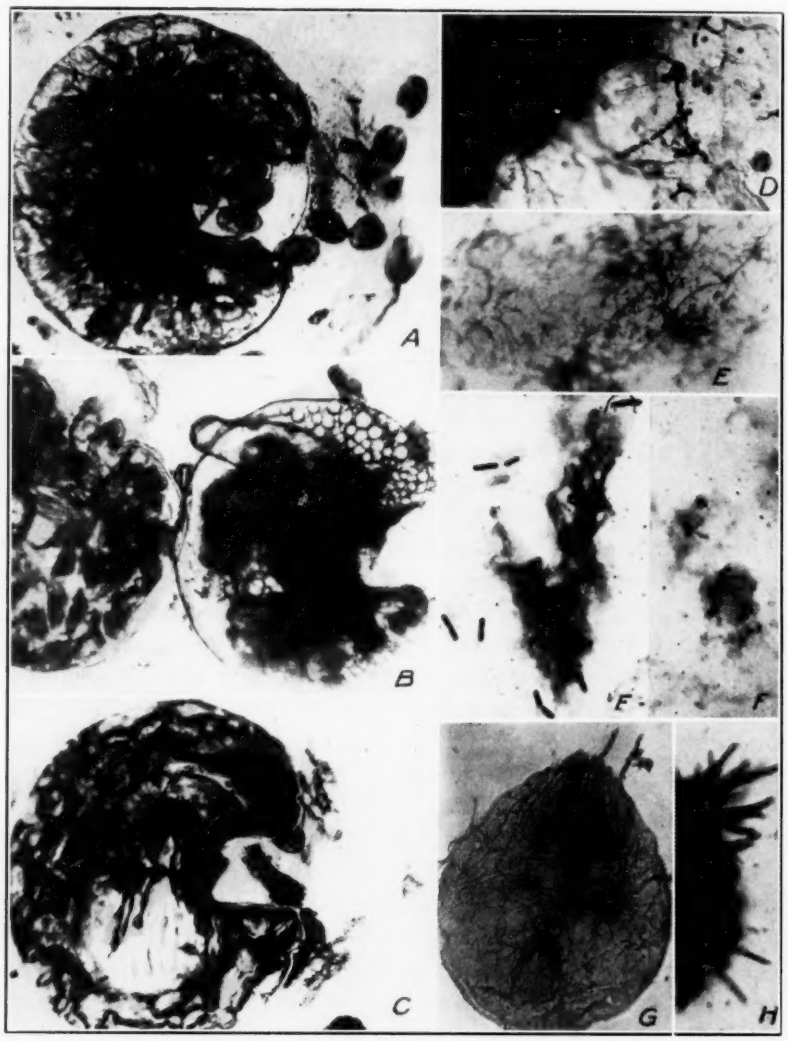

FIG. 2. A. Normal adult female citrus red scale with crawlers (ventral view). ×64. B. Infected adult female with dead crawlers (ventral view). Formation of gas within the insect. ×64. C. Final disintegration of the insect. ×64. D. Bacillus "C" invading the outer margin of the insect (Gram stain). ×1,000. E. Bacillus "C" inside the infected insect (smear preparation; Gram stain). ×250. F. Bacillus "C" in the crushed contents of the insect (Gram stain). ×1,500. G. Fungus (*Cladosporium* sp.) conidiophores growing from body of red scale (still alive). ×64. H. Conidiophores. ×140.

After 5 or 10 days of incubation, it is frequently possible to observe a general disarrangement of the internal structure of the insect. Gas bubbles

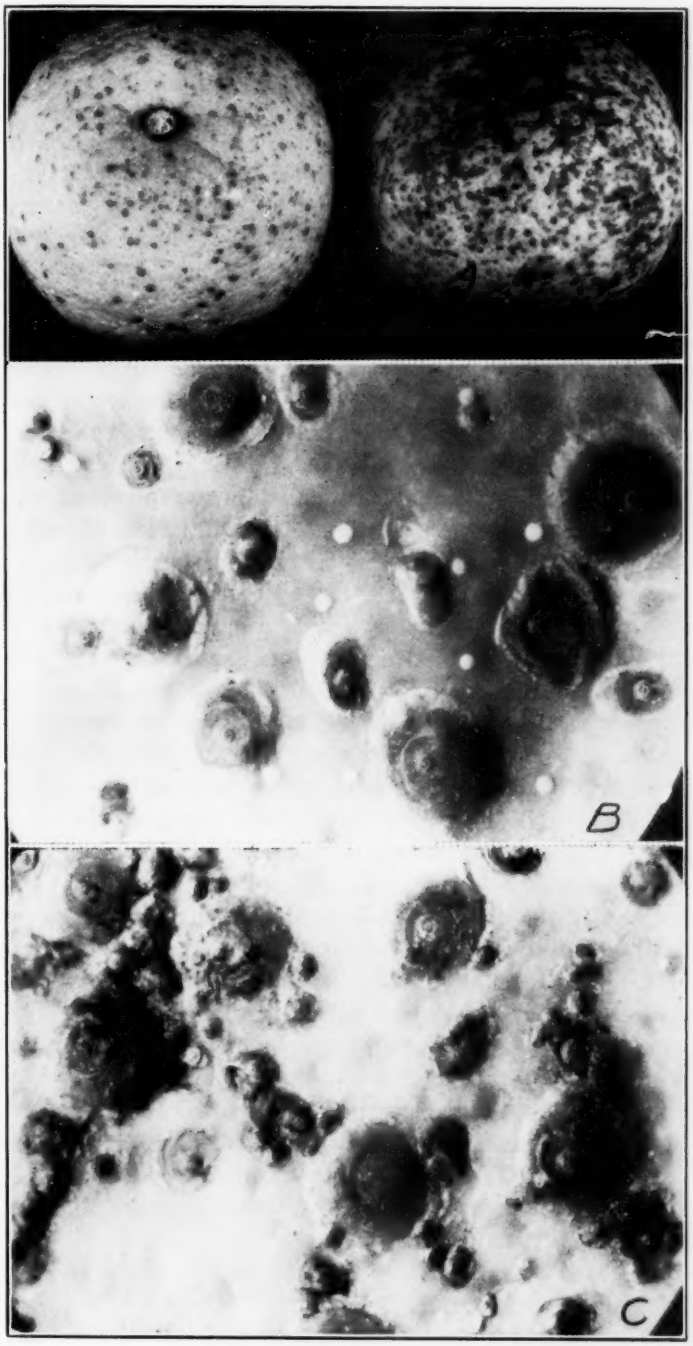

FIG. 3. Red scale on oranges: A. Effect of 16-hour immersion in a culture of bacillus "C" and of subsequent dry incubation at 28° C. for 2 weeks (right); parallel untreated control (left). B. Surface of the untreated control orange (note the white caps). C. Surface of the treated orange. ×14.

can sometimes be seen under the microscope as they are evolved from the infected body. At this stage the saprophytic fungi (facultatively parasitic) may invade the insect, unless special precautions were taken to minimize their numbers on the fruit (Fig. 2, G and H). Despite the disarrangement and apparent decomposition, the body of the insect appears relatively turgid. The drying of the dead insects takes place gradually and requires approximately 3 weeks.

There is a good deal of deviation from the course of the disease and death here tentatively charted. Some individuals, although obviously infected, exhibit but one or none of the symptoms. Others pass the decomposition stage before any browning is visible. In other words, a satisfactory definition of the symptoms of the infection or an establishment of a sequence of the successive stages of the disease leading to the death of the insect cannot yet be made with precision. There appears to be a considerable degree of variability in the nature and order of symptoms and stages, not only in the insects on different hosts but in the insects on one and the same fruit or leaf, often situated within a few millimeters of each other. It was found possible, nevertheless, by examining large numbers of adult insects systematically, to make certain comparisons between the healthy and the diseased, on the basis of their appearance under the microscope (Table 1). A summary of these comparisons includes insects from lemons, oranges, grapefruit, and a relatively small number from lemon leaves. In these comparisons, no attempt is made to differentiate between the symptoms observed on various hosts or to allow for the variations in the method of the infection (by dipping, immersion, or spraying), as it appears for the present that small differences due to these variables are overshadowed by individual variations between the insects on the same host.

After the infected red scale has been incubated for several days at 28° C., it is possible, in the smear preparations, to demonstrate abundant growth of the bacillus within the insect. Generally speaking, during the first few days of the infection single cells of the bacillus are preponderant in the invaded insect. At a later period an abundance of chains may be noted. Sporulation has been observed at all times, becoming prominent 1 or 2 weeks after the infection.

Thus far we have no evidence of phagocytosis. Nor is there yet any evidence of morphologically different stages of the bacillus, once it becomes established within its host. The microorganism appears morphologically stable or true to its form and manner of growth under a variety of the environmental conditions (Fig. 2).

IMMERSION EXPERIMENTS

While it is possible to bring about individual infection of red scale by the bacillus, regardless of the latter's age, by applying copious quantities of the bacterial suspension to individual insects by means of a loop or a pipette, successful mass infection appears to be contingent upon a number of considerations:

Motile cultures of the bacillus are preferable to nonmotile. On aerobic plates, as well as in liquid media, the bacillus becomes largely nonmotile after 1 or 2 days. Young cultures were used accordingly in all our experiments. In the instance of the aerobic plates, the most satisfactory motile cultures were obtained on 4 per cent Liebig's Extract of Beef (manufactured by Oxo Ltd., London, Eng.).

Satisfactory wetting of the fruit with the bacterial culture or suspension presents a problem of some difficulty. This mechanical difficulty may be overcome eventually by use of a suitable wetting agent. Such wetting agents as blood albumin or casein offer a degree of promise, but some that are most effective appear to be incompatible with the bacillus. In our preliminary studies, therefore, we preferred to obtain the desired result by prolonging the period of immersion rather than by the use of wetting agents.

Too rapid a drying of the treated fruit militates against successful mass infection of the insects. By extending the period of the immersion to several hours, however, this difficulty is rendered insignificant. In other experiments in which the contact of the fruit with the bacilli was relatively brief, an almost complete failure of the mass infection was observed when the fruit was allowed to dry within less than 2 hours' time.

Finally, periodic reisolations of Bacillus "C" from sterile soil[6] were found desirable to maintain the ability of the bacillus to destroy the scale. A somewhat arbitrary criterion served as a sign of the need for reisolation, namely, failure of the pure culture of the organism to grow throughout liquid media in the presence of potassium nitrite. Its ability to grow anaerobically in the presence of nitrite was commonly restored upon its reisolation. There was likewise a parallelism between its pathogenicity to the red scale and its ability to employ nitrite as a hydrogen acceptor.

Immersion for 1 or 2 hours at 28° C. was often sufficient in itself to infect and destroy every one of the adult females on lemons, regardless of the total number on a single fruit. Our preliminary studies were limited nevertheless to the 16-hr. immersion treatment as a method of ascertaining pathogenicity of the bacillus to the citrus red scale.

A summary of the results of these treatments is given in table 2. Death or survival of the insects was ascertained at the end of 2 to 4 weeks' incubation following the immersion. The exact time of the count was largely determined by the "controls." A large-scale emergence of crawlers and the subsequent appearance of "white caps" on the control fruits (there were no active crawlers or "white caps" on the treated fruits) was taken as indication of the timeliness of the comparative counting. In determining the survival of the insects, movement of the pygidia alone was not regarded as a sufficient basis. Any insect appearing free from the signs of the decomposition was deemed "living." On the other hand, any insect showing

[6] Five g. of garden soil moistened with 5 cc. of water were sterilized at 16 lb. pressure in cotton-stoppered test tubes for several successive 1-hr. periods, 2 days apart, until proved sterile by incubation with peptone-yeast broth. Soil extracts prepared in a number of different ways could not be substituted successfully for the soil.

TABLE 2.—*Mortality of adult red scale on lemons after immersion for 16 hours in cultures or suspensions of Bacillus "C"*

Treatment	Total adults	Mortality		
		Low	High	Average
Bacillus "C" in tap water	432	98	100	99
Sterile tap water only	460	36	79	55
Bacillus "C" in peptone water	652	98	100	99
Sterile peptone water only	553	35	89	61
None	887	29	60	37

signs of drying or disintegration, whether or not accompanied by browning or distortion of the pygidia, was regarded as dead.

Our understanding of the pathogenicity of the bacillus to the red scale is complicated by the fact that the fruits were not and could not be made sterile at the time of the experiment. For this reason, the so-called treated controls are not controls in the true sense of the word. In many instances, when these controls were found to harbor bacteria apparently identical with Bacillus "C," the main difference between the treated and the control fruits, under conditions of the immersion, might have been merely in the size of the inoculum. On the other hand, it is conceivable that the lethal effect of the bacillus may be enhanced by some other biological factors indigenous to the fruit or the insect. Further work may serve properly to ascertain the existence and relative importance of such factors.

DISCUSSION

It is not possible to suggest at this time any definite mechanism underlying the pathogenic effect of the bacillus on the insect, or to surmise the nature of the toxic principles at work. The data presented here may, nevertheless, provide a basis for a speculative discussion of at least one of the many possibilities in this regard.

Aqueous extracts of normal adult insects contain, as a rule, measurable quantities of nitrates. The nitric nitrogen constitutes somewhat less than 10 per cent of the total nitrogen.[7] Extracts of infected insects contain an appreciably lower amount of nitrate. The order of magnitude of the change in the nitrate concentrations is indicated in table 3.

It is evident from the available data that the slight increase in the soluble nitrite, in consequence of the infection, is insufficient to account for the loss of nitrate. Colorimetric measurements of ammonia in comparable extracts offered no evidence of any regularly significant change in the extractable ammonia of the insects. While there is as yet no direct basis to suggest

[7] Total nitrogen in the normal adult red scale from lemons was found to be 1060 and 1050 micrograms N per 100 insects. Nitric nitrogen constituted 104 and 110 micrograms N per 100 insects. For strictly comparable lots of the insects infected with the bacillus, the figures were, in micrograms N per 100 individuals, 1020 and 1020 for total N, and 47 and 45 for nitric N. The live weight of 100 normal adults is approximately 23 milligrams; of 100 dead adults it is approximately 18 milligrams.

Haas (6) reports more than 6 per cent total N in the red-scale insects, dry basis. Our results imply a slightly higher figure.

the possibility of the well-known reaction between the nitrite and the alpha amino acids of the insect, this possibility should be at least considered. Nor can we disregard the potential toxicity of the substances that may be formed in the course of the further reduction of nitrates, once this reduction could be proved to take place in the infected insect.

Immersion of lemons supporting the scale in buffered distilled water suspensions of Bacillus "C" was often accompanied by the appearance of measurable quantities of nitrite in the suspensions. These suspensions were free from both nitrate and nitrite before the fruit was introduced, and there

TABLE 3.—*Determinations of nitrate and nitrite in aqueous extracts of normal and infected adult red scale on lemons*

Insects[a]	Number of analyses	Nitrate (micrograms N)			Nitrite (micrograms N)		
		Low	High	Average	Low	High	Average
Normal	13	90	135	105	0.0	3.0	0.6
Infected with Bacillus "C"	7	0	85	56	0.5	2.8	2.0
Dead and dry, 4 weeks after the infection	6	0	35	12	0.0	0.3	0.1

[a] Tested in lots of 100.

was neither nitrate nor nitrite in the controls where only buffered distilled water was used. In view of a considerable variability in the amounts of nitrite found in the experiments of this type (ranging from a trace to several parts per million), it is not feasible, for the present, to regard this observation as an aid in the interpretation of our other results.

The nitrate content of insects subjected to hydrogen cyanide fumigation or exposed to natural illuminating gas in sealed desiccators does not differ from that of untreated insects. After 5 days of storage over pyrogallol in sealed desiccators, on the other hand, the extractable nitrate was in comparison with that of untreated insects (185 and 172 micrograms as against 100 micrograms N per 100 adult insects). Since the insects derive their nourishment from the albedo cells of their host, containing, among other things, nitrate,[8] its presence in their body fluids is scarcely surprising. The remarkable ability of the scale to survive for more than 24 hours in the absence of oxygen (4) may not be unrelated to the presence of nitrate in their substratum. Since they cannot survive indefinitely, however,

[8] The presence of nitrate in the extracts of both albedo and flavedo cells of lemons was demonstrated by the brucine and the phenoldisulphonic-acid methods, as well as by the m-xylenol method (7). Significant quantities of ammonia were formed, also, upon the addition of zinc and ferrous sulphate to ammonia-free extracts (previously distilled in the presence of alkali). Because of the well-known limitations of all these methods, when applied to plant material, only the order of magnitude of the concentrations of nitrate in the albedo cells can be indicated. It appears to be in the vicinity of 60 to 100 p.p.m. N. Grapefruit albedo cells contain much less nitrate.

under such conditions, we may venture a speculation that, while nitrate itself may not be toxic to the insects, the products of its reduction may be toxic, directly or indirectly. For example, hydroxyl amine is a known inhibitor of the cytochrome-A_3 (2). There is a similarity in the appearance of the insects that died in consequence of their infection with Bacillus "C" and of those that died after several days' storage over the pyrogallol, as well as of those whose death followed their brief immersion in hydroxyl amine.

SUMMARY

A denitrifying bacillus, unidentified with any of the known microorganisms, is able, under laboratory conditions, to invade and destroy the adult red scale on lemons. Infection and death of the scale is accompanied by a significant decrease in soluble-nitrate content. It is possible that the lethal effect of the bacillus is related to the reduction of nitrate inside the insect.

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LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 5th ed. 757 p. Assoc. Off. Agr. Chem. (Washington). 1940.
2. CRAIG, RODERICK, and W. M. HOSKINS. Insect Biochemistry. Ann. Rev. Biochem. 9: 617–640. 1940.
3. DUTKY, S. R. Two new spore-forming bacteria causing milky disease in Japanese beetle. Jour. Agr. Res. [U. S.] 61: 57–68. 1940.
4. EBELING, WALTER. Effect of oil spray on California red scale at various stages of development. Hilgardia 10: 95–125. 1936.
5. ELEMA, B. De Bepaling van de Oxydatie-Reductiepotantiaal in Bakteriencultures en hare beteekensis vor de Stofwisseling. (Delft). 1931.
6. HAAS, A. R. C. Relation between the chemical composition of citrus scale insects and their resistance to hydrocyanic acid fumigation. Jour. Agr. Res. [U. S.] 49: 477–492. 1934.
7. TRESCHOW, CECIL, and E. K. GABRIELSEN. Zur Bestimmung von Nitrat in Pflanzen und Böden. Zeitschrift für Pflanzenernähr., Düng. u. Bodenk., 32: 357–376. 1933.

PRESTORAGE DISINFECTION OF NARCISSUS BULBS

FREEMAN WEISS, FRANK A. HAASIS, AND C. E. WILLIAMSON

(Accepted for publication June 26, 1941)

The control of narcissus basal rot, caused by one or more specialized forms of *Fusarium bulbigenum* Cke. and Mass., in regions where environmental factors during the growing and bulb-storage seasons are conducive to this disease, requires periodic sanitizing treatments of the bulbs sufficient at least to prevent the superficial contamination and latent infections with this organism from becoming active infections (1, 3, 4, 5, 7, 8). For the purpose of bulb sanitation, chemical treatments might be given either at planting time, which follows the summer storage period, or between the time of harvesting the bulbs and their placement in storage (3, 4). Practical considerations, especially the greater susceptibility of the recently harvested bulbs to chemical injury, make the pre-planting application the preferable and, in commercial bulb culture, the only time for giving these treatments. Generally, a brief immersion in a suspension or a solution of one of the organic mercury disinfectants is employed in commercial narcissus culture in the United States (4, 8).

When a thermal bath is required to disinfest the bulbs of animal parasites, such as nematodes, larvae of narcissus flies, and mites, the mercury-dip treatment is supplanted by a combination warm-water and chemical treatment, for which formaldehyde often is used in place of mercury compounds because of its demonstrated effectiveness against the bulb nematode, *Ditylenchus dipsaci* (Kühn) Filipjev (1, 3). Marked improvement in health and productiveness of treated bulbs has generally resulted from these treatments, and commercial narcissus growers have acquired enough experience in the adaptation of treating dates and concentrations to the requirements of particular varieties so that chemical injury to the bulbs seldom occurs.

As basal rot is primarily a storage disease it would seem *a priori* that a sanitizing treatment applied at the beginning of the storage period should be more effective than one given prior to planting in the preceding autumn, which antedates the storage period by 8 to 10 months. Experiments have indeed shown that basal rot during storage can be almost entirely prevented by previous chemical treatment, but usually at the cost of more or less damage to growth and flowering in the subsequently planted bulbs. This injury occurs in two forms or degrees of severity, (a) that immediately visible in discolored and indurated bulb scales or necrotic pitting especially of the root plate, and (b) deformity of the flower and sometimes of the shoot during the ensuing growth of the bulb. The shoot malformation simulates the injury caused by ethyl mercury phosphate in wheat and corn seedlings (2, 6), and the floral deformity in narcissus is so characteristic that a defi-

nite diagnosis of mercury injury to the bulbs usually can be made from inspection of the flowers. Because of this flower injury, growers have seldom been willing to adopt prestorage treatments, even though the alternative risk of losing untreated bulbs from storage rot is also great. However, experimental efforts have been continued to adapt prestorage chemical treatments of narcissus bulbs to the conflicting requirements of (a) prompt application following harvesting in order to prevent superficial infections from becoming deep-seated, and (b) minimal toxicity to the bulbs, susceptibility to which declines rapidly after the bulbs become dry.

A variety of materials thought to have value for this purpose have been tested at different times during the last 10 years, but the indications were consistently in favor of mercury compounds as affording the best protection from basal rot. With the choice of materials somewhat narrowed, a comprehensive experiment was planned to bring out significant differences, if possible on a quantitative scale, between materials having different toxic components.

Apparently healthy bulbs of the leading commercial variety, King Alfred, were selected July 28, 1938 (3 days after harvesting), from a uniform stock in which about 12 per cent of the bulbs showed basal rot when dug. After thoroughly mixing the selected bulbs, 132 random samples of 50 bulbs each were drawn. Chemical treatments with different materials, concentrations and durations were applied to different samples (Table 1), there being 4 replicate samples (200 bulbs) in each treatment. The various materials were made up in aqueous solutions or suspensions and Pyrolen M–P, a commercial wetting agent, was added to each solution at a 1 to 800 dilution.

Immediately after treatment the bulbs were placed in wire-mesh-bottomed trays, which were then stacked in the open air following a random arrangement of treatment samples in 4 replicate blocks. The stacks were supported about 1 foot above the ground and were covered with gable-roofed metal covers in accordance with commercial practice in the Long Island, N. Y., narcissus district. Two examinations for basal rot were made, the first on September 7, the second on October 2. The commercial storage period for this variety as grown in Long Island generally terminates about the middle of September. In most samples there was only a slight increase, and in many none, in the number of rotten bulbs between the two examinations. The bulbs that still appeared sound on second inspection were planted out to observe the effect of the treatment on subsequent growth and flowering.

The rot count per sample and the means for the different treatments are shown in table 1.

In the analysis of the data as here presented, the significance of the differences between treatments is postulated in relation to the error variance of the data as a whole, though it is evident that the error variance of the first 4 is less than that of the remaining treatments (actually 9.786 compared with 13.035). As the object of the experiment was a general comparison

TABLE 1.—*Effect of treating narcissus bulbs with various kinds of fungicides on the development of basal rot during storage. (The figures show the number of rotten bulbs in each unit sample of 50.)*

Chemical	Concentration	Period of immersion	Replications				Means of treatments
			1	2	3	4	
	Percent	*Minutes*					
Mercuric oxide (HgO)	1.0	2	18	9	10	9	11.50
" chloride	0.2	5	16	10	9	11	11.50
" "	"	15	2	10	11	13	9.00
" " (acidulated[a])	"	5	8	8	9	5	7.50
" " "	"	15	10	4	7	10	7.75
New Improved Ceresan[b]	0.4	2	7	6	5	4	5.50
" " "	"	5	3	2	6	2	3.25
Cuprocide[b]	1.0	2	20	19	20	24	20.75
Coposil fungicide[b]	1.0	5	28	21	21	28	24.50
" "	"	15	22	22	21	15	20.00
Auragreen[b]	1.0	5	19	17	20	18	18.50
"	"	15	21	20	18	21	20.00
Stantex Helione Yellow[b]	1.0	5	30	18	18	18	21.00
" " "	1.0	15	15	21	19	26	20.25
Stantex Elgetol[b]	0.2	5	15	16	22	17	17.50
" "	"	15	15	12	24	17	17.00
Lime sulphur (dry)	1.0	5	19	25	17	24	21.25
" " "	"	15	18	14	18	19	17.25
Stantex Colloidal Sulphur[b]	1.0	5	21	27	19	21	22.00
Acetic acid (glacial)	1.0	5	19	8	10	10	11.75
" " "	"	15	14	11	13	14	13.00
Formalin (37 per cent HCHO)	1.0	5	17	14	20	11	15.50
" " " " "	"	15	18	14	7	15	13.50
Chlorox[b]	3.0	15	15	20	21	16	18.00
Phenol	0.5	5	21	17	18	22	19.50
"	"	15	16	10	12	16	13.50
Stantex Fungicide F–X[b]	0.2	5	21	20	22	16	19.75
" " "	"	15	18	19	19	16	18.00
Shirlan D[b]	0.4	5	16	19	21	21	19.25
" "	"	15	17	17	18	24	19.00
Silver cyanide	0.2	5	22	18	9	20	17.25
" "	....	....	25	34	25	21	26.25
Control	"	15	21	17	20	20	19.50

Difference of 4.93 between means of treatments = 5 per cent level of significance.
" " 6.54 " " " " = 1 per cent level of significance.

[a] Contained 1 per cent acetic acid.

[b] The essential ingredients and the sources of these materials were as follows: New Improved Ceresan (5 per cent ethyl mercury phosphate)—Bayer-Semesan Co., Inc.; Cuprocide (98 per cent cuprous oxide)—Röhm & Haas Co., Inc.; Coposil (ca. 16 per cent Cu)—California Spray Chemical Corp.; Auragreen—Mallinckrodt Chemical Works; Helione Yellow, Elgetol (22 per cent sodium dinitro ortho cresylate), colloidal sulphur and Fungicide F–X—Standard Agricultural Chemicals, Inc.; Chlorox (5 per cent sodium hypochlorite); Shirlan D (salicylanilide)—Imperial Chemical Industries.

between the different chemical types, as mercury, sulphur, copper, etc., compounds, and not a close comparison between members of these groups, the segregation of error variance was not attempted. A slight obscuration of possibly significant differences between certain treatments may result, but the major conclusions as follows are not affected:

(1) A number of the treatments produced statistically significant decreases in the number of bulbs rotting in storage.

(2) Only acidulated mercury bichloride and New Improved Ceresan resulted in commercially important control of rot.

(3) The results with copper and sulphur compounds were not indicative of their prospective value for this purpose.

The New Improved Ceresan treatment for durations of 2 and 5 minutes caused both bulb and flower injury, the latter affecting about 90 per cent of the flowers produced. In all other treatments the condition of the flowers was equal to that in the checks. Since a similar treatment, applied at planting time, is an established practice in commercial narcissus culture, further work was undertaken to find means of reducing or avoiding the flower injury that results from prestorage applications.

Experience had shown that flower injury from treatment with ethyl mercury compounds preceding storage could be reduced by delaying the treatment for 1 to 2 weeks after harvesting, during which period the bulbs became appreciably more dry and firm. As previously mentioned, the reduction in phytotoxic effects was accompanied by a serious loss in efficiency of rot control, the amount of rot becoming equal to that in non-treated samples when the treatment was deferred for 2 weeks. In an effort to fix an intermediate point at which a minimum of flower injury occurs while the fungicidal benefits are preserved, a second experiment, immediately following the first, was conducted, using the same bulb stock and sampling methods. Different samples were immersed in New Improved Ceresan solution, composed of 1 lb. of chemical in 32 gal. of water, for 2, 5, or 10 minutes on the third day after digging. Similar sets of samples were treated at intervals of 6, 9, 12, and 15 days after digging. The treated bulbs were placed immediately in wire-bottomed trays to dry in the open air, and thereafter stored in 4 replicate blocks with a modified Latin square arrangement of samples. Despite their exposure in this way, the bulbs remained wet for irregular periods, and the projected 2-minute immersion was prolonged in effect so as to approximate the longer treatments. The results as regards control of basal rot are shown in table 2.

It is evident from the figures showing the number of bulbs rotting after treatment for 2, 5, or 10 minutes that the duration of treatment had but little effect on its efficiency in preventing rot. Thus the total numbers of bulbs rotting in *all* samples treated for 2, 5, and 10 minutes are respectively 306, 286, and 263, and the corresponding means are 15.3, 14.3, and 13.2. On the other hand, the total numbers of bulbs rotting in all samples treated at each of the 3-day intervals after digging are 62, 109, 205, 212, 267, showing a progressive increase in rot from the first 3-day interval to the fifth. The corresponding means are 5.17, 9.08, 17.08, 17.67, 22.25 and each difference is significant, except between the 3rd and 4th intervals. Since the duration of the treatment had no significant effect on control of rot, mean differences between the 2-, 5-, and 10-minute treatment periods were not computed but these data were combined to show the highly significant differences due to the period of delay in treating.

As before, the remaining healthy bulbs were planted out to observe the effect of the treatment on growth and flowering (Table 3). Flower injury

TABLE 2.—*The development of basal rot of narcissus bulbs in storage following treatment with New Improved Ceresan at successive intervals of 3 days between time of digging and time of treatment. All treatments in a solution of 1 lb. of chemical to 32 gal. of water*

Interval between harvest and treatment	Replicates	No. of bulbs per sample of 50 that rotted in storage after treatment for			Mean for treatment interval
		2 min.	5 min.	10 min.	
Days	*Number*				
3	1	7	3	8	
	2	6	2	5	
	3	5	6	8	
	4	4	2	6	
	Mean	5.5	3.25	6.75	5.17
6	1	14	9	3	
	2	12	8	10	
	3	6	14	7	
	4	7	11	8	
	Mean	9.75	10.5	7	9.08
9	1	21	16	14	
	2	24	20	19	
	3	11	14	19	
	4	15	15	17	
	Mean	17.75	16.25	17.25	17.08
12	1	13	17	12	
	2	20	23	18	
	3	20	18	14	
	4	21	22	14	
	Mean	18.5	20	14.5	17.67
15	1	23	25	17	
	2	32	19	16	
	3	22	18	25	
	4	23	24	23	
	Mean	25	21.5	20.25	22.25
Mean for immersion period		15.30	14.30	13.15	

Difference of 2.735 between means of treatments = odds of 19:1
" " 3.657 " " " " " " 99:1

was manifestly severe, being nearly total in the bulbs treated 3 days after digging, and marked in all the others up to the 15-day interval, when it became practically 0. Furthermore, significant differences in flower injury, in contrast to the results on rot control, appeared in the bulbs treated for different periods from 2 to 10 minutes. Thus, in the 9-day treatment interval, the 2-minute duration caused flower injury in only about 10 per cent of the bulbs as contrasted with about 68 and 95 per cent injury in the 5- and 10-minute durations. It may be seen in table 2 that the 2-minute treatment on the 9th day after digging caused a significant reduction in rot which, in

TABLE 3.—*Flower injury resulting from bulb treatments given before storage as listed in table 2*

Interval between harvest and treatment	Replicates	Flower injury after treatment for			Mean for treatment interval
		2 min.	5 min.	10 min.	
Days	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
3	1	100	88	100	
	2	100	100	100	
	3	100	100	100	
	4	74	44	79	
	Mean	93.5	83.0	94.8	90.4
6	1	38	92	100	
	2	87	100	100	
	3	90	79	96	
	4	89	100	100	
	Mean	76.0	92.8	99.0	89.3
9	1	5	88	100	
	2	10	94	91	
	3	14	68	95	
	4	15	21	100	
	Mean	11.0	67.8	96.5	58.4
12	1	4	63	62	
	2	30	47	100	
	3	24	69	84	
	4	22	33	92	
	Mean	20.0	53.0	84.5	52.5
15[a]	1	0	0	0	
	2	0	0	8	
	3	0	0	0	
	4	0	0	0	
	Mean	0	0	2	00.7
Mean for immersion period		40.1	59.3	75.4	

[a] Data from the 15-day interval were not used in the variance analysis.

Significance involving day interval

Difference of 4.489 between means of intervals = odds of 19 : 1
" " 6.036 " " " " " " 99 : 1

Significance involving periods of immersion

Difference of 3.888 between means of periods of immersion = odds of 19 : 1
" " 5.227 " " " " " " 99 : 1

terms of mean number of rots per sample of 50, amounts to 18 as compared with 25 for treatment on the 15th day and (from Table 1) 26 for no treatment at all.

There are circumstances when even this 31 per cent reduction in rot would be more important than the associated 11 per cent flower injury; for example, when a grower is building up a stock of valuable novelties, but for commercial purposes the control of rot would have to be improved to the level of the 3rd or 6th day treatment without the correlated increase in flower injury. This might be achieved by reducing the concentration of chemical or, in view of the previous observation that delay in natural drying

of the bulbs after treatment may invalidate the regulation of the treating time, by drying the bulbs artificially.

Experiments have been undertaken employing both of these modifications. In them, as well as in commercial-scale tests by several growers in Long Island, New York, it was found possible to prevent basal rot in storage to a high degree and without encountering any visible bulb injury. The actual flowering records of the treated bulbs have not yet been obtained, and publication will, therefore, be deferred.

SUMMARY

Chemical sanitizing treatments of narcissus bulbs applied prior to the period of summer storage have been found to effect a marked reduction in basal (Fusarium) rot during the storage period.

Of the various kinds of fungicidal materials thus far tested, certain mercury compounds, particularly ethyl mercury chloride and ethyl mercury phosphate, have proved most effective. With these materials, a 2-minute immersion of the bulbs is equal to longer treatments up to 10 minutes, in protecting against rot.

Treatments applied soon after the bulbs are dug, more effectively prevent rot than do those applied later. The protective effect is practically lost in treatments delayed for more than 2 weeks.

Both of the ethyl mercury compounds cause flower-bud injury, manifest in the subsequent production of crippled flowers, when the treatments are given too soon after the bulbs are dug. This injury increases with the time of immersion and diminishes with the length of interval between digging and treating, becoming negligible after a delay of 2 weeks.

A practicable compromise between the conflicting requirements of adequate protection against rot and minimization of flower injury is foreshadowed but has not definitely been reached.

LITERATURE CITED

1. BEAUMONT, A. The use of chemicals in the hot treatment of narcissus bulbs. Seale-Hayne Agr. Coll. Pamphl. 49: 22–28. 1938.
2. CROSIER, WILLARD. Abnormal germination in dusted wheat. Phytopath. 24: 544–547. 1934.
3. HAWKER, LILIAN. Experiments on the control of basal rot of narcissus bulbs caused by *Fusarium bulbigenum* Cke. and Mass. Ann Appl. Biol. 27: 205–217. 1940.
4. MCWHORTER, FRANK P., and FREEMAN WEISS. Diseases of narcissus. Oregon Agr. Exp. Stat. Bull. 304. 41 pp. 1932.
5. MILES, L. E. Control of basal-rot of narcissus. Mississippi Agr. Exp. Stat. Tech. Bull. 19. 12 pp. 1932.
6. SASS, JOHN E. Histological and cytological studies of ethyl mercury phosphate poisoning in corn seedlings. Phytopath. 27: 95–99. 1937.
7. SLOGTEREN, E. VAN. Warm-waterbehandeling van narcissen en bolrot. Weekbl. Bloembollencult. 42 (15): 130–135. 1931.
8. WEISS, FREEMAN. The control of basal rot of narcissus bulbs. Florists Exchange 80 (14): 9, 11. 1932.

THE CONIDIAL PHASE OF SCLEROSPORA NOBLEI

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In 1929 the writer (11) described *Sclerospora noblei*, a new species, which had been found by R. J. Noble of the Department of Agriculture of New South Wales, occurring apparently endemic under natural conditions in Australia as a destructive parasite on *Sorghum plumosum* Beauvois (*Andropogon australis* Sprengel), a native grass locally known as "wild sorghum," of value as an abundant wild fodder for sheep and horses. At that time only the resting spore phase of this parasite was known; but as this, on comparison with other *Sclerosporas*, especially *Scl. sorghi* (Kulk.) Uppal and Weston (13), the only one whose resting spores had been reported on any sorghum previously, proved to have adequate distinguishing characters, the species was established on this basis. Naturally, however, the writer hoped that its conidial, or perhaps sporangial, phase might be discovered so that our knowledge of the species might be extended and a more adequate basis for comparison might be available. Since then Dr. Noble has found the conidial phase and this, from material that he very generously sent the writer, proves, on comparison with other species, to be distinctive also. The purpose of the following note, therefore, is to supplement the previous paper by describing the conidial phase and noting the points of interest it presents.

SEASONAL OCCURRENCE, SYMPTOMS, AND DEVELOPMENT

The wild-sorghum host is perennial and, in the cooler regions of Australia, its new growth starts in September to October, the beginning of their summer. The conidial phase of the *Sclerospora* begins to become apparent soon after and continues for some months, material being collected by Dr. Noble as late as December, January, and February.

The symptoms, like those of other systemic conidial *Sclerosporas*, involve the development, on successively unfolding leaves, of pallid, linear streaks from which, under favorable conditions, the downy growth of conidiophores and conidia emerges. Like other conidial *Sclerosporas* also, the development of the conidiophores and conidia takes place under natural field conditions during the early hours of the morning after the leaves have been covered for some time by the nocturnal condensation of dew. In the summer months in Australia Dr. Noble noted this nocturnal production of conidia between 3 and 5 a.m., not only in the field but also in clumps transplanted to the garden of his home near Sydney.

By February the ensuing oogonial or resting-spore phase is already apparent on some infected clumps, the noticeable shredding of the leaves,

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 193.

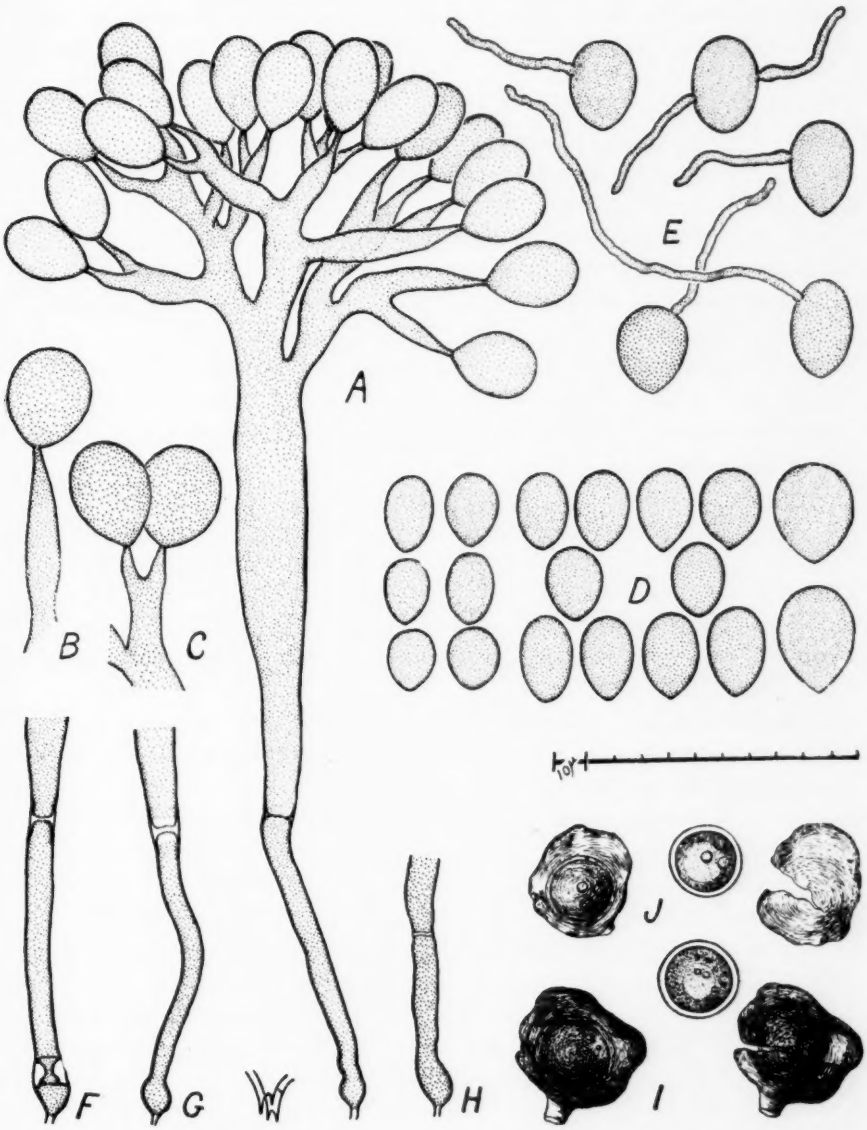

FIG. 1. Conidial phase of *Sclerospora noblei*. Camera lucida drawings from material preserved by Dr. Noble. Magnification $\times \pm 375$. *Cf.* scale. A. Typical conidiophore with bulbous footed basal cell, expanding main axis, and extensive branch system bearing numerous conidia of average size. B–C. Conidia of maximum size *in situ* showing the range in size and shape of the sterigmata from the single, long, slender branch ending of B to the short pair of C. D. Representative conidia, the middle 10 showing the predominant sizes of 25–30.9 × 20–22.9 μ, the 6 at the left the small extreme, the 2 at the right the large extreme. E. Germinating conidia showing typical germination by hyphae. F–H. Basal cells showing commonly encountered variations in size and structure. I–J. Resistant spores, at left intact showing the difficulty of discerning the characteristics of the enclosed oospore through the enveloping oogonial wall, and at right revealing these characteristics in the oospore squeezed out after treatment with acid.

so characteristic of this phase, becoming increasingly obvious throughout the remainder of the season.

CHARACTERISTICS OF THE FUNGUS

The conidiophores show the species to be allied to the destructive, predominantly conidial, *Sclerosporas* of the Orient, for they are luxuriantly developed and arbusculate, 300–450 μ in height, with a basal cell above whose septum extends the trunk-like main axis bearing the primary branches (usually 3) of approximately equal size and extent, which branch and rebranch more or less dichotomously to form an elaborate system whose ultimate tips bear the conidia (Fig. 1, A).

The basal cell, with its somewhat swollen or knobbed foot attached by a slender hypha through the stomata to the lobed clump of intramatrical hyphae in the substomatal chamber, extends upward, sometimes isodiametric, but usually expanding, its diameter from 8 to 13 μ, usually around 10 μ, its length from 68 to 110 μ most commonly about 90 μ, thus occupying about $\frac{1}{3}$ to $\frac{1}{2}$ of the complete trunk and $\frac{1}{6}$ to $\frac{1}{4}$ of the total height of the conidiophore (Fig. 1, A). The septum, which delimits the basal cell from the main axis, may be a complete cross wall (Fig. 1, G, H) or a modification of the longitudinal wall substance in the form of a ring-like thickening (Fig. 1, F) that slightly or noticeably constricts the lumen. Rarely is more than one encountered (Fig. 1, F).

The main axis expands gradually, reaching its greatest diameter of around 28 μ (20–34 μ) at a point about $\frac{1}{2}$ to $\frac{2}{3}$ its length beyond the septum, then remaining approximately isodiametric to the emergence of the branches, its length (exclusive of the basal cell) ranging from 120 to 200 μ but usually about 170 μ. The branch system is well developed and extensive, ranging from 70 to 130 μ in height and 100 to 130 μ in spread, and comprising 2 to 4 primary branches that give rise to the secondary, tertiary, or, in rare cases, quaternary sets, terminating in the tapering sterigmata, 10 to 15 μ long, that bear the conidia. The sterigmata vary in length and basal thickness, depending on whether they are the short terminations of tertiary branchlets (Fig. 1, C) or the longer endings of secondary branches (Fig. 1, B). In all cases they taper to ultimate tips of about 2 μ diameter on which the conidia are borne. As a result of the spreading branch system the conidia are disposed over a somewhat parasol-shaped or hemispherical area.

The conidia vary in number in proportion to the extent and luxuriance of the branch system, being most commonly 24 to 48, rarely more, occasionally on depauperate conidiophores with reduced branch system as few as 6 or 8.

The conidia show considerable variation in shape, but are usually obovoid with the base tapering slightly to the point of attachment and the distal end bluntly rounded, the greatest diameter being a little more than half way to the tip (Fig. 1, D).

The conidia are true conidia, the wall being thin and nearly equal throughout and absolutely lacking any thickened, modified papilla for dehiscence. In consequence, the method of germination is by hyphae, usually one, very rarely more (Fig. 1, E).

All the foregoing features, when taken together, contribute toward the characterization of the species, but no one of them in itself is particularly salient or diagnostic.

The size of the conidia, however, is a dependable characteristic, which effectually distinguishes the conidial phase of *Sclerospora noblei*. In this, as in other species of the genus, the conidial size shows considerable range, but serves as a reliable means of comparison and distinction when based on measurements of suitable numbers that express quantitatively the frequency of various size classes.

The size characteristics of the conidia of *Sclerospora noblei* are presented in table 1.

TABLE 1.—*Size measurements of the conidia of Sclerospora noblei on Andropogon australis in New South Wales, Australia. From preserved material collected by R. J. Noble*

Length		Diameter	
Classes in μ	No. of conidia in 200	Classes in μ	No. of conidia in 200
21–22.9	10	13–14.9	2
23–24.9	18	15–16.9	8
25–26.9	41	17–18.9	36
27–28.9	38	19–20.9	43
29–30.9	52	21–22.9	60
31–32.9	18	23–24.9	21
33–34.9	20	25–26.9	22
35–36.9	2	27–28.9	7
37–38.9	1	29–30.9	1

These measurements are from conidia collected by Dr. Noble during the period of optimum conidial production betwen 3 and 5 a.m., immediately killed in suitable fluid, and sent to the writer in preservative. The material was in excellent condition, well fixed and free from plasmolysis, but it should be noted that in the case of other species the writer, on comparing the measurements of long-preserved conidia with those of fresh conidia mounted in dew and measured immediately, has found that the preserved material usually shows a slight shrinkage.

The size frequencies as here tabulated make no pretense at being a precise statistical study, for such is not necessary to bring out the salient characteristics. It is obvious from the table that, while the length varies from the extremes of 21 to 38.9 μ, and the diameter from 13 to 30.9 μ, the range of size most frequent and representative was more restricted, 149 out of 200 being between 25–32.9 μ in length, 103 out of 200 between 19 and 22.9 μ in diameter. In size characters, therefore, they lie between the smaller, rotund-

spored forms such as *Sclerospora maydis* (Rac.) Palm (3) on maize in Java, with lengths most commonly between 17 and 24.9 μ, or *Scl. sorghi* (Kulk.) Weston and Uppal (13), on sorghum in India, with lengths between 19 and 24.9 μ, and the elongate-ellipsoidal-spored form *Scl. philippinensis* Weston (7) of maize in India and the Philippines, with most common lengths between 31 and 36.9 μ.

Thus *Sclerospora noblei* presents an interesting combination of characteristics; its resting-spore phase distinguished by its relatively small oospores and especially by its oogonial wall, dark, closely adherent to the enclosed oospore and unusually thick, its bluntly rounded protrusions—not outbulgings but actual thickenings to as much as 20 μ; its conidial phase distinguished by the intermediate shape and size of the conidia, which place this species between the forms with small, rotund conidia and those with large, elongate-ellipsoidal conidia. Both phases are regularly formed on the same host plant, the conidial beginning early in the growing season and lasting a few months, the oogonial appearing later and persisting until growth of the host ends for the season.

Adequate characterization of *Sclerospora noblei* now necessitates extending the diagnosis, previously based on the resting-spore phase alone, to include the distinguishing features of the recently discovered conidial stage. Also, further investigation has revealed that the previous description of the resting spore phase must be emended slightly with respect to the thickness of the oospore wall. Through the thick, dark, oogonial wall of these spores it is difficult to measure accurately the exact wall thickness of the enclosed oospore. Consequently, the writer has tried various methods for freeing the oospores of this and other species by mechanical cracking or chemical softening of the oogonial wall. Of these, treatment with acids, as suggested to the writer by Dr. E. J. Butler in 1930, has proved most practicable. When, for example, the resting spores of *Scl. noblei* are treated with concentrated nitric acid for 5 to 10 minutes, washed, and mounted, slight pressure on the cover glass will crack the oogonial walls, freeing the oospores intact (Fig. 1, I and J). These are apparently uninjured, and their diameter, 23 to 28.9 μ for 82 out of 100, agrees with that of the 600 previously measured *in situ*. Their wall thickness, however, is much greater than the 1 to 1.5 μ previously recorded, for, in 76 out of 100, this ranges from 1.75 to 3.25 μ. Further investigation is necessary to determine how much of this is actual and how much the possible effect of the acid.

Diagnosis: Sclerospora noblei.[1]

Conidiophoris 300–450 μ altis, cellula basali inferne in bulbo inflata 68–110 μ, plerumque circa 90 μ longa, 8–13 μ, plerumque circa 10 μ crassa; trunco 120–200 μ, plerumque circa 170 μ longo, leniter expanso, ad latitudinem maximum 20–34 μ, plerumque circa 28 μ, in regioni ½–⅔ altitudinis supra septum; parte summa arboris 2–4ies dichotome ramosa, ramulis divergentibus; sterigmatibus conoideo-subulatis 10–15 μ long.

Conidiis obovoideis, 21–38 × 13–30 μ, plerumque 25–32.9 μ long., 19–22.9 μ cr., hyalinis, episporio tenui, apice sine papilla ergo semper per tubum germinantibus.

Oogoniis ovoideis vel subsphaeroideis 28–44 μ diam., membrana fusco-flavida vel castaneo-brunnea saepe fere opaca, plerumque 5–10 μ, rare 3 μ, saepe ad 20 μ, crassa.

[1] *Sclerospora noblei* sp. nov.

Oosporis sphaeroideis, 20–34 μ, plerumque 23–28.9 μ diam. hyalinis vel pallido flavidis, episporio 1–3 μ crasso, intus granulosis, guttulis oleosis praeditis. Germinatio non visa.
Hab. in foliis *Andropogonis australis* Sprengel, in N. S. Wales, Australia.

Conidiophores well developed, 300 to 450 μ in height, comprising basal cell, main axis, and spreading branch system. Basal cell with knobbed or swollen foot, then slightly expanding or more rarely isodiametric, usually about 10 μ (at times 8 to 13 μ) in diameter extending for a length of 68 to 110 μ (usually about 90 μ) to the delimiting septum, which is usually complete but may be a partial ring-like thickening. Main axis expanding gradually above the septum, reaching its greatest diameter of usually about 28 μ (20 to 34 μ) at a point about ½ to ¾ its length beyond the septum, then retaining this diameter to the first branches, its length exclusive of the basal cell 120 to 200 μ, usually about 170 μ. The branch system well developed and extensive ranging from 70 to 130 μ in height and from 100 to 130 μ in spread with 2 to 4 large primary branches, then secondary, tertiary, or rarely quaternary ones; terminating in tapering sterigmata usually about 10–15 μ long, which bear the conidia, the whole forming a subdichotomously deliquescent branch system so arranged that the conidia are spread over a parasol-shaped or hemispherical area.
Conidia obovoid ranging from 21 to 38.9 μ by 13 to 30.9 μ, most frequently 25 to 32.9 μ by 19 to 22.9 μ. Conidia hyaline with thin, continuous, unmodified wall and granular content, germinating invariably by hyphae.
Resting spores (as previously described) showing rather obviously that they comprise a modified oogonium with oospore within. Oogonium usually ovoid, ellipsoid, pyriform or subspherical in shape, occasionally with bluntly rounded projections rendering it gibbous and unsymmetrical, ranging in diameter from 28 to 44 μ. Oogonial wall closely adherent to the oospore within, the exterior protrusions not representing outbulgings, but rather involving actual increases in thickness of the wall, which most commonly is 5 to 10 μ, at times only 3 μ, often as much as 20 μ. Wall dark, often scarcely transparent, color ranging from somewhat golden (Mars yellow Ridg.) to rich-brown (Brussels brown Ridg.), most commonly dark resinous amber (Sudan brown Ridg.). Fragment of oogonial stalk frequently adherent. Oospores regularly spherical, most frequently from 23 to 28.9 μ in diameter, the mode between 25 to 26.9 μ, extremes ranging from 20 to 34 μ; wall hyaline to pale golden, 1 to 3 μ thick; content finely granular with aggregations of denser material and with masses of oily reserve substance, central to eccentric in position.
Germination of the resting spores not yet observed.

Both phases occurring on *Sorghum plumosum* Beauv. (*Andropogon australis* Sprengel) in New South Wales, Australia, collected by R. J. Noble. The conidial phase occurring on pallid streaks on the leaves in summer (Dec., Jan., Feb.), developing as a downy outgrowth at night when the leaves are wet with dew; the successive oogonial phase beginning in Jan. and Feb. and continuing to develop throughout the growing season, causing the disintegration of the interfascicular tissue and the fraying of the leaves into withered tangled shreds.

DISCUSSION

Now that our knowledge of *Sclerospora noblei* has been amplified by the discovery and study of the conidial phase, further points of interest merit consideration.

The relationship of this species is now clarified, its conidia with finality precluding any alliance with the sporangial (potentially zoospore producing) *Sclerospora graminicola*, and definitely indicating association with the series of species characterized by true conidia. In this series the size and shape of its conidia place *Scl. noblei* in an intermediate position between the group with relatively small, rotund conidia (*e.g.*, *Scl. maydis* and *Scl. sorghi*) and the group with larger, more elongate-ellipsoidal conidia (*e.g.*, *Scl. philippinensis* and *Scl. sacchari* [1]).

Moreover, *Scl. noblei* regularly develops both its conidial and its resting-spore phase successively on *Andropogon australis*, a point of resemblance to

Sclerospora sorghi, which forms both phases on *Sorghum vulgare*, but a distinct contrast to *Scl. maydis*, *Scl. philippinensis* and *Scl. spontanea* (8), which, on maize, an introduced host, form only the prominent conidial phase, but are suspected of developing both phases on wild grasses within their geographic range, and a point of disagreement with such species as *Scl. northi* on *Erianthus* in the Fiji Islands (10) and *Scl. butleri* on *Eragrostis* in Nyasaland (12), in which the resting spore phase seems the predominant one or at least the only one as yet known.

Furthermore, *Sclerospora noblei* is distinctive and specifically characteristic in both its conidial and its resting-spore phases, and can be identified with certainty in either. This distinctive individuality of both phases is in marked contrast to the situation in *Scl. sorghi* and *Scl. graminicola*, which, although absolutely distinct from each other in their respective conidial or sporangial phases, are very difficult to distinguish in their resting-spore stages.

In the second place, in the development of its conidial phase *Sclerospora noblei* corroborates the correlation of conidiophore production in *Sclerospora* under natural field conditions with the nocturnal suffusion of the leaf surface with dew or other moisture, a correlation first formulated by the writer (9) in the case of *Scl. philippinensis* and *Scl. spontanea*, and since confirmed in the case of other species by various investigators, most recently by Steyaert (4).

Finally, *Sclerospora noblei*, since its discovery by Dr. Noble ten years ago, has been found only on the one original host, *Andropogon australis* (2). During this time no evidence of its transfer to other hosts in the field has ever been encountered nor was Uppal (13) in his cross-inoculation studies with *Scl. graminicola*, *Scl. sorghi*, and *Scl. noblei* able to secure infection by *Scl. noblei* on *Andropogon sorghum*, *Pennisetum typhoideum*, *Setaria italica*, *Euchlaena mexicana*, and 3 varieties of *Zea mays*, even though he used the same technique of inoculation by resting spores in the soil that has proved notably successful in the case of other species. Possibly after the vicissitudes of their journey from Australia to India the resting spores may not have been germinable; in any case, such negative results in a relatively small number of tests can be regarded only as indicative rather than conclusive.

Yet, despite the foregoing evidence, it does not seem safe to the writer to dismiss *Sclerospora noblei* as of no potential menace to valuable cultivated gramineous crops, for the cumulative evidence from successful cross inoculations with other species (*cf.* 5, 6) seems to justify regarding any *Sclerospora* occurring on members of the Andropogoneae as possibly dangerous to cultivated Gramineae. Indeed, this species must now be considered as potentially even more dangerous than before, because its conidial phase, although not producing the vast quantities typical of *Scl. philippinensis* on maize (9), develops formidable numbers of infective conidia in successive crops for months during the growing season.

SUMMARY

The writer's original characterization of *Sclerospora noblei* on its distinctive resting-spore phase is here supplemented by the description of its equally distinctive conidial stage more recently collected by Dr. Noble. The well developed, arbusculate conidiophores and the true conidia ally this species with the predominantly conidial species of the Orient; the size of the conidia, most commonly from 25 to 32.9 μ by 19 to 22 μ, together with the relatively small oospores and dark, unusually thick oogonial wall previously noted, distinguishing this species.

The species is compared with others and such points of interest are noted as the regular occurrence of both reproductive phases on the same host, the development of the conidiophores at night when the plants are wet with dew, and the apparent limitation of this parasite to its indigenous Australian grass host.

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LITERATURE CITED

1. LYON, H. L. The Australian leaf stripe disease of sugar cane. Hawaiian Plant. Rec. **12**: 257–265. 1915.
2. NOBLE, R. J., H. J. HYNES, F. C. MCCLEERY, and W. A. BIRMINGHAM. Plant diseases recorded in New South Wales. Dept. of Agr. N. S. Wales Sci. Bull. **46**: 47 p. 1934.
3. PALM, B. Onderzoekingen over de Omo Lyer van de Mais. Med. van het Lab. voor Plantenziekten. **32**: 1–78. 1918.
4. STEYAERT, R. L. Presence du *Sclerospora maydis* (Rac.) Palm (*S. javanica* Palm) au Congo Belge. Publ. Inst. Nat. Étude Agron. Congo Belge. Sér. scient. 13: 16 p. 1937.
5. UPPAL, B. N. A new host of *Sclerospora graminicola* var. *andropogonis-sorghi*. Internat. Bull. Plant Protect. **5**: 26. 1931.
6. ———, and M. K. DESAI. Physiologic specialization in *Sclerospora graminicola* (Sacc.) Schroet. Indian Jour. Agr. Sci. **2**: 667–678. 1932.
7. ———, and WM. H. WESTON. The basis for merging *Sclerospora indica* with *Scl. philippinensis*. Indian Jour. Agr. Sci. **6**: 715–719. 1936.
8. WESTON, WM. H. Another conidial Sclerospora of Philippine maize. Jour. Agr. Res. [U.S.] **20**: 669–684. 1921.
9. ———. Production and dispersal of conidia in the Philippine Sclerosporas of maize. Jour. Agr. Res. [U.S.] **23**: 239–278. 1923.
10. ———. A new Sclerospora from Fiji. Phytopath. **19**: 961–967. 1929.
11. ———. A new Sclerospora from Australia. Phytopath. **19**: 1107–1115. 1929.
12. ———. A new Sclerospora from Nyasaland. Phytopath. **23**: 587–595. 1933.
13. ———, and B. N. UPPAL. The basis for *Sclerospora sorghi* as a species Phytopath. **22**: 573–586. 1932.

VARIETAL RESISTANCE TO BLOSSOM-END ROT IN TOMATOES[1]

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(Accepted for publication June 25, 1941)

Physiological blossom-end rot has caused large losses each year in spring tomatoes (*Lycopersicon esculentum* Mill.) in eastern Texas. Severe loss from blossom-end rot in most commercial fields of tomatoes in 1937 apparently resulted from abundant rains, promoting succulent growth of the plants, followed by unusually long periods of drought. Despite certain drought periods in the 3 succeeding years, rains were abundant and distributed well enough to promote large yields of fruit; blossom-end rot caused relatively little loss in most of the fields of Marglobe and Rutgers tomatoes.

REVIEW OF LITERATURE

Brooks (1) reported blossom-end rot resulting from both drought and excess water. He described blackened placentae near the blossom-end of fruit with normal external appearance. Stuckey (5) found that resistance of tomatoes to blossom-end rot is inherited. Higgins (3) stated that blossom-end rot of pepper is caused by drought following a period of rapid growth of plants and fruit. Under such conditions, wilting leaves may remove water from cells in the blossom-end of fruits. More recently, Foster (2) found that high-nitrogen plants were very susceptible to blossom-end rot, whereas applications of superphosphate decreased the amount of this abnormality. Wedgworth (6) also presented data showing the relative amounts of blossom-end rot in several varieties of tomatoes; and the writer (7) classified recent commercial varieties as resistant or susceptible to this trouble.

MATERIALS AND METHODS

In this work, the fields in which the plants were set were plowed in November, and 600 lb. per A. of a 6–10–7 fertilizer were distributed in the rows late in March. Tomato seeds treated with red or yellow cuprous oxide were planted in hot beds about February 10, and the seedlings were transplanted 4 in. apart in cold frames about March 5. Early in April, when the plants were 6 to 12 in. tall, they were transplanted from cold frame to field, usually in units of 24 plants each. The plants were set 21 in. apart in rows 5 ft. apart. Each plant was pruned to one main stem and tied to a stake. The fields were cultivated frequently with sweep blades, from April to June, to control weeds and to keep the soil crust broken. All plants received a side dressing of 200 lb. 6–10–7 fertilizer per acre about May 7 each year, except for differences in side dressing fertilizer, as noted elsewhere. Fruits with blossom-end rot were picked and recorded every 7 to 10 days.

[1] Approved as Technical Contribution No. 760 by the Director of the Texas Agricultural Experiment Station.

TABLE 1.—*Amount of blossom-end rot in tomato varieties, 1937 to 1939, inclusive*

Variety	No. of plants	Blossom-end rot; number of fruits per plant			Variety	No. of plants	Blossom-end rot; number of fruits per plant		
		Min.	Max.	Ave.			Min.	Max.	Ave.
Baltimore	517	0.2	2.1[c]	0.9	Louisiana Pink[b]	1082	0.2	2.2	0.9
Bay State	81	0.1	.....	.....	Louisiana Red[b]	1183	0.5	3.6	1.6
Blair Forcing[b]	530	0.0	0.2	0.1	Marglobe[b]	3503	0.0	1.3	0.2
Break O'Day[b]	603	0.1	0.7	0.4	Marhio[b]	320	0.1	0.7	0.3
Brimmer	63	0.7	.....	.....	Marvana[b]	373	0.2	2.3	0.7
Brown's Special	90	0.8	3.4	2.0	Marvel[b]	214	0.2	1.0	0.5
Buckeye State[b]	317	0.7	6.1	1.9	Marvelosa	204	0.6	3.9	1.8
Century[a]	402	0.2	2.6	0.9	Michigan State[b]	528	0.1	0.8	0.5
Columbia	229	0.3	2.2	0.8	New Invincible	188	0.1	1.0	.....
Duke of York	166	0.1	.....	.....	Newport 4 and 5[b]	396	0.2	1.0	0.4
Early Baltimore[b]	259	0.0	1.0	0.4	Norton	137	0.2	0.7	.....
Everbearing Scarlet Globe	72	0.2	.....	.....	Norduke	81	0.4	.....	.....
Extra Early Prolific	138	1.0	.....	.....	Oxheart	66	0.3	0.7	.....
Garraprihe[a]	104	0.0	1.3	0.2	Pearson	92	0.1	0.3	.....
Globe (Livingston, Landreth)	301	0.2	0.4	0.3	Prairiana[b]	349	0.5	2.9	2.1
Globelle 1 and 2	159	2.4	3.9	3.1	Pritchard[b]	794	0.0	0.6	0.2
Globelle 3	70	1.0	.....	.....	Red Cherry	66	0.0	.....	.....
Glovel	112	1.5	2.0	.....	Redfield Beauty	36	0.6	.....	.....
Golden Queen	92	.....	1.1	.....	Riverside[a]	331	2.0	5.0	3.6
Grothen's Red Globe[b]	392	0.2	1.3	0.6	Rutgers[b]	2467	0.1	4.0	0.9
Gulf State Market[b]	958	0.2	3.8	1.1	Summerset	207	0.0	1.2	0.4
Illinois Baltimore[a]	471	0.2	2.5	0.9	Sureset Forcing[b]	362	0.2	0.9	0.5
Illinois Pride[b]	233	0.3	2.9	0.6	Sweetmeat[a]	151	0.3	2.8	1.6
Indiana Baltimore	64	0.7	2.4	.....	Tennessee Red[a]	83	0.2	.....	.....
Invincible	172	0.7	1.6	.....	Urbana Forcing	69	0.3	.....	.....
Kanora[a]	155	0.1	1.0	0.9	"White-flowered" selection	801	0.0	0.5	0.3
Lloyd Forcing	90	0.1	.....	.....	Yellow Ponderosa	60	0.1	0.3	.....
Long Calyx Forcing[b]	289	0.0	0.3	0.1					
Louisiana Dixie[a]	144	0.3	4.7	2.8					
Louisiana Gulf State	45	0.3	.....	.....					

[a] Variety tested 2 years. [b] Variety tested 3 years. [c] Variation in numbers of fruits with blossom-end rot per plant was due mainly to variation in fields and to combining many selections of varieties in summarizing data.

The plants were sprayed with calcium arsenate and copper fungicides to control insects and diseases attacking the leaves and fruit.

The data in table 1 were obtained from plants growing in Ironton red soil that was abundantly infested with the tomato-wilt fungus, *Fusarium lycopersici* Sacc., but it was practically free from the root-knot nematode, *Heterodera marioni* (Cornu) Goodey (8). Consequently, blossom-end-rot data were recorded only for the varieties that showed time-weighted wilt resistance of at least 40 per cent. Wilt-susceptible varieties, including Bonny Best and Stone, were omitted from the table because the plants died of wilt before sufficient information was secured as to their susceptibility to blossom-end rot.

EXPERIMENTAL RESULTS

As recorded in table 1, the following varieties showed the most resistance to blossom-end rot: Blair Forcing, Break O'Day, Grothen's Red Globe, Long Calyx Forcing, Marglobe, Marhio, Marvana, Marvel, Michigan State, Newport, Pritchard, Surest Forcing, and "White-flowered" (9) selections. The resistance of the Pritchard, Marglobe, and "White-flowered" selections was evident also when the plants were grown in soil practically free from *Fusarium lycopersici*. Thus, fusarium wilt showed no apparent effect on the resistance of the tomato varieties to blossom-end rot described in table 1. This may be explained by the fact that plants with moderate or severe symptoms of fusarium wilt may suffer from inadequate water, even when growing in moist soil. Foster (2) has stated that plants growing continuously with low soil moisture appear to be resistant to blossom-end rot.

Comparative data on blossom-end rot in different varieties were secured also from tomatoes grown in fields free from soil-inhabiting parasites. Amounts of blossom-end rot were calculated separately for groups of rows of Marglobe (Landreth Certified) tomatoes without sprays and for groups of rows separately sprayed with 8 copper sprays each year. The plants were grown in fields of Norfolk fine sandy loam soil, practically free from *Fusarium lycopersici* and *Heterodera marioni*. In the 4-year period, the

TABLE 2.—*Relation of blossom-end rot to yield of tomatoes—1940*

Tomato variety	No. of plants	Number of blossom-end-rot fruits per plant	Tons of marketable fruit per acre
Buckeye State	129	5.0[a]	0.879
Grothen's Red Globe	122	6.7	6.825
Gulf States Market	132	7.6	7.694
Illinois Baltimore	132	7.1	6.497
Louisiana Red	128	17.2	5.688
Marglobe	127	2.9	8.339
Pritchard	125	1.2	8.762
Rutgers	133	7.1	6.898
"White-flower" selection	135	2.1	7.717

[a] Before June 5th, Buckeye State variety bore too few fruits to show its susceptibility to blossom-end rot.

groups of plants showed 0.1 to 1.7 fruits with blossom-end rot per plant. There was no correlation between the amount of blossom-end rot and differences in the copper-spray formulae. The Marglobe tomatoes regularly showed strong resistance to blossom-end rot, with only a narrow range in the

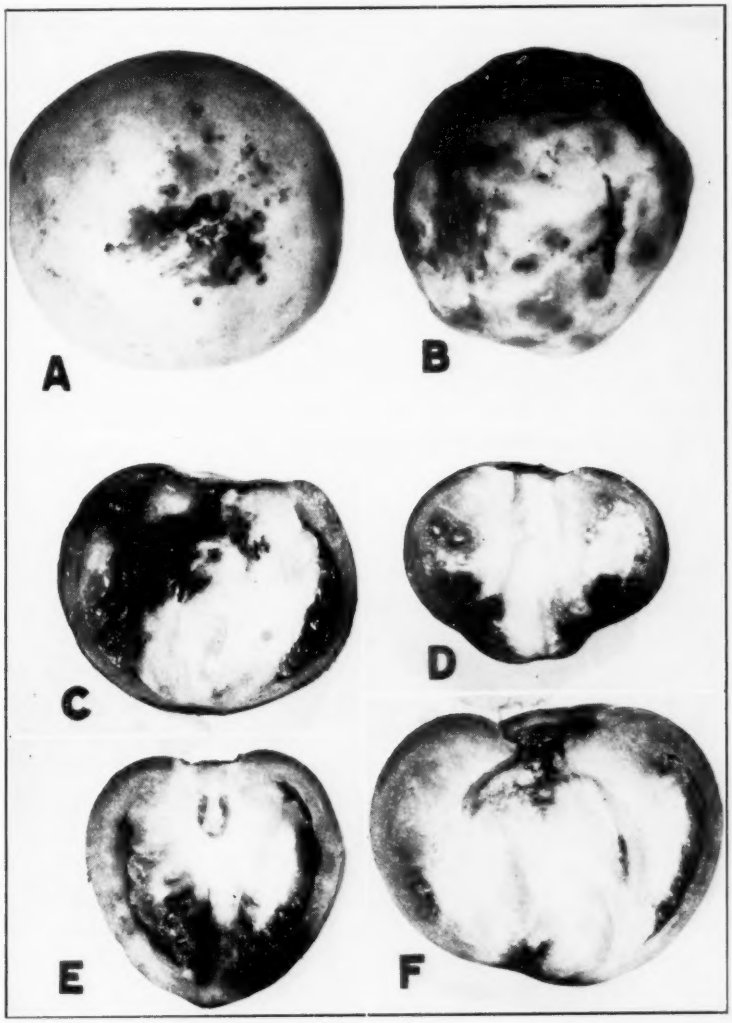

FIG. 1. Symptoms of blossom-end rot of tomatoes. A. Ordinary early externally visible symptoms consisting of brown or black blotches in peel. The following unusual symptoms are illustrated: B, brown depressions indicating deep necrotic tissues in fruit; C, D, E, necrotic areas in fruits showing superficial brown depressions; F, vascular browning near stem end associated with blossom-end rot, and possibly related to physiological core rot.

amounts of the disease in the different years. This range was similar to that for Marglobe selections described in table 1.

Table 2 compares the amounts of blossom-end rot and yields of fruit of 9 varieties of tomato, each growing in 6 randomized plots of Norfolk fine

sandy loam, practically free from soil-borne parasites. The plants were set on April 4, 1940, and the records on blossom-end rot were first taken on May 25. The disease was more severe in Marglobe tomatoes growing in this part of the field with very light sandy soil receiving 10–0–10 fertilizer in the side dressing, than in the adjacent part with heavier sandy soil and 6–12–6 fertilizer in the side dressing. The difference may be due to the lower amount of phosphate and the lower water-holding capacity of the soil in the former part of the field. Although blossom-end rot decreases tomato yields, the lower yields were not closely correlated with the numbers of diseased fruits per plant. Such a correlation apparently is affected by differences in the fruit-setting tendencies of the different varieties.

Also, in 1940, 100 plants of Buckeye State tomato, in a field of Ruston soil, averaged 3.9 fruits with blossom-end rot per plant with the same fertilizer treatment as that described for the plants in table 2. In the other experimental fields, the many varieties averaged less than 0.1 fruit with blossom-end rot per plant during this same year.

The first externally visible symptom of blossom-end rot in eastern Texas usually appears as light-brown or abnormally dark-green, apparently water-soaked spots, $\frac{1}{16}$ to $\frac{1}{2}$ in. wide in the peel of the blossom-end of tomato fruits $\frac{1}{2}$ to 3 in. in diameter. At first, the spots are limited to the subepidermal cells (Fig. 1, A). Later, the necrotic lesion enlarges until it involves deep tissues and the spot becomes a sunken, brown area with a distinct margin. Many of the small fruits become hemispherical or more extremely flattened from shrinkage of the lower part of the blossom-end-rotted fruit, a condition especially noticeable in the fruit of Louisiana Red and Buckeye State varieties. Areas with blossom-end rot often are rotted by species of *Alternaria* and *Fusarium*. In addition to these symptoms, an unusual type of the disease appeared during rainy weather in June, mainly in Buckeye State tomatoes. These fruits had uneven surfaces with brown areas, indicating internal brown tissues (Fig. 1, B–E).

The number of fruits with blossom-end rot per plant gives only an estimate of decreases in calculated yields, because varieties of tomatoes differ much in the number of fruits formed, and in the percentage of their fruits that develop the disease while they are very small. Estimates are useful, however; so the following method of calculation was tried in 1939: Six bushels of green-wrap tomatoes contained 1370 individual fruits with an average weight of 3.35 oz. each. With the usual 4978 plants per acre, loss of one blossom-end-rotted fruit per plant gave a calculated loss of $\frac{1}{2}$ ton of fruit per acre. Blossom-end rot rarely causes greater loss of Marglobe tomatoes. Using a variety with ordinary susceptibility, *e.g.*, Rutgers, 143 plants produced an average of 4 large end-rotted fruits per plant, or a calculated loss of 2 tons of fruit per acre. A like loss was seen in 5 acres of the same variety in which blossom-end rot ruined more than half of the large yield of fruit.

Resistance to blossom-end rot may be stronger in one strain of tomato than in another strain of the same variety. For example, T708 Rutgers was

more resistant than T638 Rutgers, and T50 Marvelosa was more resistant than T57 Marvelosa.

CONTROL

Blossom-end rot is a physiological abnormality, attributable probably to a varietal weakness. Accordingly, the relative resistance to blossom-end rot may be related to the extent and efficiency of the root systems of different varieties of tomatoes. Although there is a certain amount of variation in the resistance to blossom-end rot in a given variety or selection in different fields and from year to year, yet the range in such variation indicates that some varieties are resistant, while others are moderately or extremely susceptible.

Fall tomatoes in the Winter Garden region of Texas are grown in fertile soil. With the usual adequate irrigation, the plants suffer little from drought. The typically large vines cover the ground and develop fruit in October when the weather becomes cool. Surveys in that locality in 1936, 1937, and 1939 showed that blossom-end rot was rare, even in the susceptible Rutgers and other varieties of tomato. This is evidence that the rot may be controlled by irrigating tomatoes enough to avoid drought injury and facilitate abundant yield of fruit. Stuckey and Temple (4) found less of the disease following irrigation.

Practical control of blossom-end rot of tomatoes without irrigation, in eastern Texas, consists mainly of planting the varieties Marglobe and Pritchard, using 600 to 800 lb. of 6–10–7 or 6–12–6 fertilizer per acre, and cultivating the plants to give them the most uniform, favorable water supply available each year. Even when the soil is moist, a day or more of warm, dry wind may cause some drought rigor in tomatoes. Shallow cultivation presumably delays the evaporation of water from wet soil, increases the oxygen available to the roots, makes the plants depend on deep roots in moist soil, and conserves water and fertilizer for the tomatoes by controlling the weeds. Tomato plants should be cultivated every 7 to 10 days, especially as soon as a soil crust forms after each rain or rainy period until most of the crop has been picked. The lightest sandy fields should be avoided, as they may favor the development of blossom-end rot.

SUMMARY

Marglobe and Pritchard are the commercial varieties strongly resistant to blossom-end rot in eastern Texas. The tests showed least blossom-rot in Blair Forcing, Break O'Day, Grothen's Red Globe, Long Calyx Forcing, Marglobe, Marhio, Marvana, Marvel, Michigan State, Newport, Pritchard, Surest Forcing, and "White-flowered" selections. Most of the other varieties tested were moderately susceptible to the disease. Riverside, Louisiana Red and Buckeye State were extremely susceptible.

Fusarium wilt had no apparent effect on the resistance of tomatoes to blossom-end rot.

An uncommon symptom of blossom-end rot is described.

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LITERATURE CITED

1. BROOKS, C. Blossom-end rot of tomatoes. Phytopath. 4: 345–374. 1914.
2. FOSTER, A. C. Effect of environment on metabolism of tomato plant as related to development of blossom-end rot of the fruit. Phytopath. 29: 7, 1939; and 27: 128, 1937.
3. HIGGINS, B. B. Blossom-end rot of pepper (*Capsicum annuum* L.). Phytopath. 15: 223–229. 1935.
4. STUCKEY, H. P., and J. C. TEMPLE. Blossom-end rot. Georgia Agr. Exp. Stat. Bull. 96. 1911.
5. ———. Transmission of resistance and susceptibility to blossom-end rot in tomatoes. Georgia Agr. Exp. Stat. Bull. 121. 1916.
6. WEDGWORTH, H. H. Investigations on diseases of vegetable and ornamental plants. Miss. Agr. Exp. Stat. Bull. 261. 1928; and Bull. 247. 1927.
7. YOUNG, P. A. Resistance of tomato varieties to blossom-end rot. Phytopath. 30: 28. 1940.
8. ———. Tomato wilt resistance and its decrease by *Heterodera marioni*. Phytopath. 29: 871–879. 1939.
9. ———. White-flower character from X-ray treatment of tomato seed. Jour. Hered. 31: 78–79. 1940.

HOST RANGE AND GROWTH-TEMPERATURE RELATIONS OF CORYNEUM BEIJERINCKII[1]

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The shot-hole disease of *Prunus* spp., caused by *Coryneum beijerinckii* Oud., has a wide geographical distribution. It is found in France, Italy, Germany, and probably in other parts of Europe, and has been reported from Asia, Argentina, Algeria, Australia, New Zealand, Mexico, and the United States.

On peaches, in California, the disease has been observed[2] to cause a shot-hole effect on the leaves and to kill the young growth, but it is not found in equal severity in all the fruit-growing districts of the State. It is reported of rare occurrence in Santa Clara County and has never been observed in Alameda County.

The host range of *Coryneum beijerinckii*, as reported by Wilson,[3] includes the following species of stone fruits: *Prunus armeniaca* L., *P. avium* L., *P. communis* Fritsch (*P. amygdalus* Stokes), *P. davidiana* Franch, *P. domestica* L., *P. laurocerasus* L., *P. padus* L., *P. persica* Sieb. and Zucc., *P. persica* var. *nectarina* Maxim., *P. serotina* Ehrh., *P. virginiana* L.

Blodgett[4] lists *Prunus emarginata* Walp. as susceptible to *Coryneum beijerinckii* in Idaho. Flachs[5] reports that *P. laurocerasus*, the cherry-laurel, is occasionally attacked in Germany by an organism closely related to *C. beijerinckii* and states that reddish-yellow to brownish spots are found on the leaves, the tissue thus spotted eventually falling out and leaving a shot-hole effect.

In southern California during the 1940–41 season, the rainfall, which occurred mostly in March and April, was approximately twice the normal amount, and conditions were especially favorable for the development of *Coryneum* on early-leafing varieties of stone fruits. The shot-hole disease was observed in plantings at the Citrus Experiment Station on leaves of *Prunus fenzliana* Fritsch (S.P.I. 27336), *P. majestica* Koehne (S.P.I. 55417), and *P. umbellata* Ell., and in commercial orchards on leaves of almond, apricot, Japanese plum (very severe), and peach. Inoculation studies for the determination of host range and temperature studies of the disease undertaken at that time are herein reported.

[1] Paper no. 447, University of California Citrus Experiment Station, Riverside, California.

[2] Smith, Ralph E., E. H. Smith, T. F. Hunt, and B. J. Jones. California peach blight. California Agr. Exp. Stat. Bull. 191: 73–98. 1907.

[3] Wilson, Edward E. The shot-hole disease of stone-fruit trees. California Agr. Exp. Stat. Bull. 608: 40 p. 1937.

[4] Blodgett, Earle. Fruit diseases reported from Idaho. U. S. Dept. Agr. Bur. Plant Ind. Pl. Dis. Rptr. 21: 215. [Mimeo.] 1937.

[5] Flachs. Schrotschusskrankheit an Kirschlorbeer. Blumen und Pflanzenbau 42: 65. 1927.

ARTIFICIAL INOCULATIONS

Artificial inoculations with *Coryneum beijerinckii* were made on different species of *Prunus*. The cultures used in the inoculations were isolated from peach, apricot, and almond. These isolates appeared to be indistinguishable as to character, and were readily cross-inoculable between different species of *Prunus*.

Spores of *Coryneum beijerinckii* were atomized onto the new growth of leaves and stems. While still wet, the stems were punctured by means of a steel needle. (The leaves were not punctured.) Some inoculations did not require protection to maintain proper moisture conditions for development of the infection; others were enclosed for 48 hours in a moist chamber or in a cellophane sack. So far as is known, no previous reports of comparative inoculations with spores of *C. beijerinckii* on *Prunus* have been made.

About 1 to 2 weeks after inoculation, typical spots appeared on the leaves. The diseased tissue soon dropped away and left the typical shot holes. The following species of *Prunus* were found to be susceptible to inoculation with *Coryneum beijerinckii*: *P. alleghaniensis* Porter,*[6] *P. angustifolia* Marsh.,* *P. armeniaca* L., *P. armeniaca* var. *mandshurica* Maxim.,* *P. avium* L., *P. besseyi* Bailey, *P. bokhariensis* Royle (S.P.I. 40223), *P. caroliniana* Ait. (*Laurocerasus caroliniana* Roem.), *P. cerasifera* Ehrh.,* *P. davidiana* Franch (S.P.I. 36664), *P. demissa* Walp. (*P. virginiana* var. *demissa* Torr.), *P. domestica* var. *insititia* Bailey,* *P. fenzliana* Fritsch (*Amygdalus fenzliana* [Fritsch] Korsh.) (S.P.I. 27336), *P. fremontii* Wats., *P. hortulana* Bailey,* *P. ilicifolia* Walp., *P. kansuensis* (*Amygdalus kansuensis* [Rehder]) (S.P.I. 68976), *P. lyonii* Sarg., *P. mahaleb* L., *P. majestica* Koehne (S.P.I. 55417), *P. maritima* Marsh,* *P. mexicana* Wats., *P. mume* Sieb. and Zucc., *P. munsoniana* Wight and Hedr.,* *P. orthosepala* Koehne,* *P. persica* Sieb. and Zucc., *P. pissardii* Koehne, *P. pseudo-cerasus* Lindl. (S.P.I. 18587), *P. reverchonii* Sarg.,* *P. salicina* Lindl., *P. serotina* Ehrh., *P. spinosa* L., *P. texana* Dietr., *P. umbellata* Ell. (S.P.I. 38974), *P. watsonii* Sarg., *P. yedoensis* Mats.,* *Prunus* spp. (S.P.I. 31652, 33217, 56121) Tunisian almond (S.P.I. 101096).

Lesions on stems caused by inoculations through puncture wounds developed on *Prunus armeniaca*, *P. kansuensis*, *P. persica*, *P. mahaleb*, *P. mira* Koehne (S.P.I. 34601), *P. munsoniana* (lesions coalesced with gum formation), *P. salicina*, and *P. yedoensis*.

While as noted by Wilson,[7] moisture is an important factor in the development of *Coryneum beijerinckii* on host plants, age of the leaves is also important. Young, succulent leaf tissue proved more favorable for spore infection than older leaf tissue. This fact was observed in many of the

[6] The species marked with an asterisk (*) were secured from the Arnold Arboretum as seed or scions.

[7] Wilson, Edward E. The shot-hole disease of stone-fruit trees. California Agr. Exp. Stat. Bull. 608: 40 p. 1937.

inoculations, especially those on apricot leaves and on leaves of *Prunus lyonii* and *P. ilicifolia*.

Leaf infections of 3 evergreen species of *Prunus*, *P. ilicifolia*, *P. lyonii*,

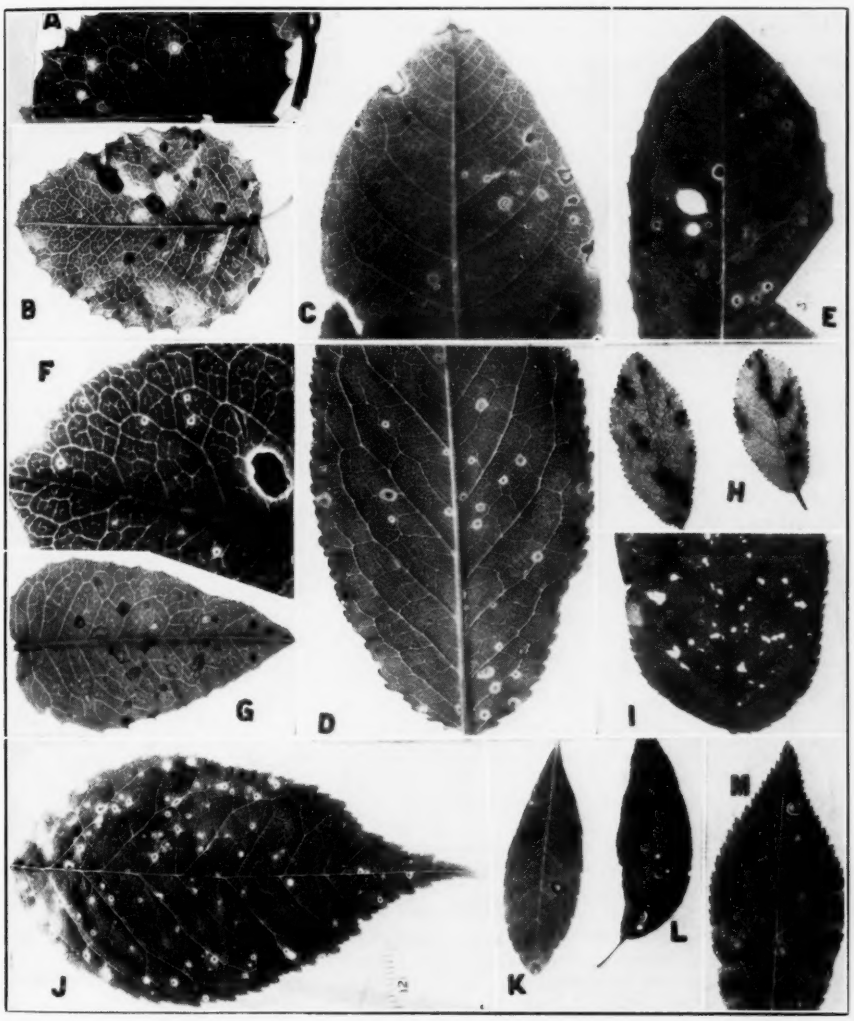

FIG. 1. Leaves of *Prunus* spp. artificially inoculated by atomizing the uninjured leaves with spores from cultures of *Coryneum beijerinckii* isolated from *Prunus* spp. A. *P. ilicifolia* (from almond) after 15 days. B. *P. ilicifolia* (from apricot) after 20 days. C. *P. demissa* (from peach) after 8 days. D. *P. orthosepala* (from *P. caroliniana*) after 6 days. E. *P. caroliniana* (from apricot) after 25 days. F, G. *P. lyonii* (from almond): F, after 45 days; G, after 6 days. H. *P. texana* (from apricot) after 14 days. I. *P. mexicana* (from peach) after 13 days. J. *P. hortulana* (from almond) after 12 days. K. *P. besseyi* (from apricot) after 19 days. L. *P. reverchonii* (from apricot) after 19 days. M. *P. maritima* (from peach) after 12 days.

and *P. caroliniana*, are shown in figure 1. These species are closely allied with *P. laurocerasus*. On *P. lyonii* (Fig. 1, F) there was one large infected area that did not fall out, and several smaller ones. The same was true of

infected areas on *P. ilicifolia*. Lesions were produced on leaves of *P. caroliniana* by infection through natural injuries, as well as through the stomata. Several of the infected areas dropped out, and the resulting shot-hole effect may be seen in figure 1, E. These 3 species and the other species of *Prunus* illustrated in figure 1, *P. besseyi*, *P. demissa*, *P. hortulana*, *P. maritima*, *P. mexicana*, *P. orthosepala*, *P. reverchonii*, and *P. texana*, are all native to the United States.

The different species of *Prunus* do not appear to be equally susceptible to *Coryneum beijerinckii*, but the data on this subject are not sufficient for

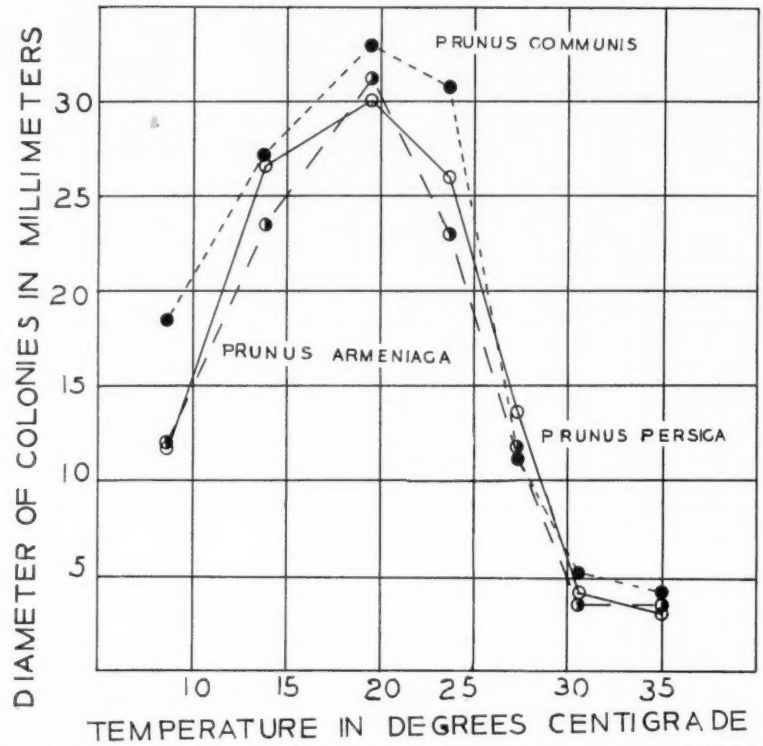

FIG. 2. Curves showing growth-temperature relations of *Coryneum beijerinckii* isolated from apricot (*Prunus armeniaca*), peach (*P. persica*), and almond (*P. communis*), when grown at constant temperatures. Optimum mycelial growth occurred at about 19° C.

comparison of susceptibility. The results of inoculation of *P. domestica*, *P. salacina*, and *P. serotina* showed only a few spots and spot holes. *P. besseyi* developed a few brownish spots 2 to 3 mm. in diameter. *P. hortulana*, *P. reverchonii*, and *P. umbellata* are extremely susceptible.

From the positive results obtained in the inoculation tests, it appears that the organism is omnivorous and that other species of *Prunus* will be susceptible under favorable conditions.

GROWTH-TEMPERATURE RELATIONS

Temperature is believed to be an important factor in the development of *Coryneum beijerinckii*. The disease appears to develop best at tempera-

tures prevalent during cool rainy weather of winter and spring in California.

Growth from pure cultures of the 3 isolates (from apricot, peach, and almond) was tested at constant temperatures of 8, 13, 19, 23, 27, 30.5, and 35 degrees C. for a period of 1 week, and measurements of the diameters of the colony were made every 2 days. The curves (Fig. 2) are similar for each of the isolations: with increase in temperatures, the growth curves became much steeper; optimum growth occurred at about 19°. Growth was considerably reduced at 27°, and none occurred at 30.5° C. (Fig. 2 and 3).

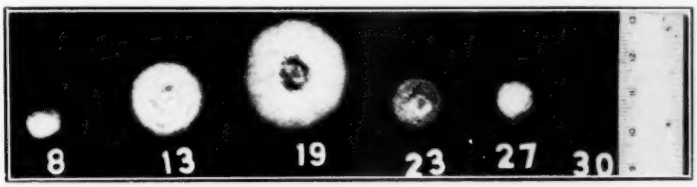

FIG. 3. Relative size of colonies of *Coryneum beijerinckii* from apricot, after 7 days' growth on glucose-potato agar at the temperature indicated (°C.).

Spore formation was observed in the colonies at the lower temperatures. A few spores were formed at 8° and 13°, and there was abundant spore formation at 19° and 23°. No spores were observed at the higher temperatures of 27° and 35° C.

SUMMARY

Inoculations with *Coryneum beijerinckii* on leaves and stems of *Prunus* spp. gave positive results of infection on about 35 species. The disease was indicated by a shot-hole effect on the leaves, although spots that did not fall out sometimes appeared in the leaf tissue. The spots appeared on the uninjured leaves in 1 to 2 weeks after inoculation.

From inoculation tests so far, it seems apparent that the organism is omnivorous in attacking species of *Prunus*. Among the species showing positive results were *P. ilicifolia*, *P. lyonii*, and *P. caroliniana*, all evergreen species under California conditions. *P. demissa*, the western choke cherry, was also susceptible.

Optimum development of the organism in culture tests occurs at about 19° C. A few spores are produced at 8° and 13°. Abundant sporulation occurs at 19° and 23°; no spores were observed at temperatures of 27° and higher.

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MEASURING MAGNITUDE OF A DEFOLIATION DISEASE OF TOMATOES[1]

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(Accepted for publication July 2, 1941)

INTRODUCTION

One of the difficult problems in plant pathology is to measure rapidly and precisely the magnitude of disease attack. Stevens (6) has recently made an earnest plea for recording disease attack, even though quantitative methods for making such measurements may not be available.

While making a quantitative study of the performance of protective fungicides on tomato foliage, it became imperative to make first a quantitative study of magnitude of infection. Otherwise, the effect of the fungicide on infection could not be measured with precision.

When dealing with a large number of field plots, it is necessary to have a rapid method that is also precise. McKinney (5) has proposed a rapid technique that depends upon grouping plants into 5 classes based on magnitude of disease. This method is less objective than desirable because it depends somewhat upon judgment. Likewise, its precision was not known. The objectiveness and precision of the method have been investigated.

MATERIALS AND METHODS

The defoliation disease of tomatoes caused by *Alternaria solani* was used as the test disease. The comparative data were obtained from a series of plots sprayed with various copper compounds where the amount of disease varied with the performance of the materials.

Counting Disease

Counting leaf spots is highly recommended, because of its objectivity, as a method of measuring magnitude of infection, but it is seldom used because it is so slow that not enough plants can be examined in an experimental plot to offset the errors due to plant variability. The objectivity is somewhat less than perfect, also, if more than one disease is prevalent or if insect punctures are present.

The number of counts can be reduced without sacrificing much objectivity if leaves are divided into 2 groups—healthy and sick, as Martin (4) has done in his research on tomato fungicides. Even this is much too slow. The fewest counts, and, therefore, the fastest method, is to divide plants into 2 groups—sick and healthy. This method has a low order of precision, however, because the number of plants required for precision at both ends of the scale cannot be provided in any reasonably-sized experimental plot.

[1] The research for this paper was begun at the New York State Agricultural Experiment Station, Geneva, N. Y., and completed at the Connecticut Agricultural Experiment Station, New Haven, Conn. It was conducted in cooperation with the Crop Protection Institute.

The McKinney Grouping Method

The McKinney (5) method is essentially a compromise in this case. As applied here to a defoliation disease, it has the rapidity of the plant-counting technique because the condition of individual plants is recorded. It has most of the precision of the leaf counting technique because each plant is ranked into 1 of 5 groups on the basis of the leaf area attacked. It loses a certain amount of objectivity, however, because the individual leaves are not examined. The amount of disease on the leaves is estimated.

The infection ratings are based, therefore, on the relative proportion of total leaf area on the individual plant killed by fungus attack. The infection categories are as follows: 0 = infection-free or nearly so, 1 = trace to 25 per cent of leaf area killed, 2 = 26 to 50 per cent killed, 3 = 51 to 75 per cent killed, 4 = 76 to 100 per cent killed. In practice 2 men should record data on any series of experimental plots. One man walks crosswise of the plots, so that they lose their identity. He calls out the category number of each plant. This is recorded by the second man on a plot map. The data are calculated by the following formula:

$$\text{Infection index} = \frac{\Sigma \text{ category numbers}}{\text{No. plants} \times 4} \times 100$$

The 4 in the denominator represents maximum infection and 100 is used to convert to percentage. In dealing with fungicides the infection index is subtracted from 100 to give percentage control, which brings the data into line with other toxicological data.

Marsh, Martin, and Munson (3) state that the grouping procedure is open to statistical analysis on the basis that "although the estimates are not necessarily in direct linear relation to the amount of fungus present . . . , they are reducible to a linear function of this amount."

The chief drawback to the method is that it is difficult to assign the same meanings to the infection categories from time to time. This, however, is not particularly troublesome to the trained observer.

Experience has shown that McKinney's 5 groups make a useful number. Marsh, Martin, and Munson (3) used 10 for a project on potato fungicides; but it seems to the writers that use of 10 groups adds more confusion by doubling the number of border-line possibilities than it adds in precision. With 5 groups the dividing lines are so far apart that errors in judgment on border-line cases are insignificant.

Yield

Yield records are usually considered close approximations to objective records, and they have found some favor as a measure of magnitude of disease. Fungicides are often compared through yield, but, since fungicides often dwarf plants, their protective value cannot always be compared through yield.

In the case of vegetables that, like tomatoes, are picked fresh, yield records are particularly difficult to assess because of the difficulty in assign-

ing a valid criterion of ripeness. Fruits on defoliated tomato vines usually come up to an orange color, seldom to the deep red that fruits attain on non-defoliated plants. Obviously, both do not come to the same stage of ripeness. As will be shown elsewhere (2), relation between disease and yield of ripe tomato fruit may be negative, absent, or positive depending upon many factors.

EXPERIMENTAL

The fungicide experiments at Geneva, N. Y., in 1938, offered an opportunity for comparing several methods of recording magnitude of infection because *Alternaria solani* attacked so frequently that the untreated plants were almost completely defoliated by mid-September. The treatments differed sufficiently among themselves to give a spread in magnitude of infection over almost the whole range.

For each treatment there were 4 replicate field plots of 20 plants each of a pure line of John Baer tomato. There were 12 treatments and a check.

The amount of stem-end rot was recorded on the ripe fruits as they were picked during the season. At the end of the season in late September, the following data were recorded (Table 1): infection index, as just described,

TABLE 1.—*A comparison of various methods of measuring incidence of defoliation on tomatoes caused by Alternaria solani at Geneva, N. Y. in 1938*

Treatment	Disease index	Wgt. of green fruit	Wgt. per green plant less fruit	Leaves killed per plant	Stem-end rot on fruit
	Per cent	*Pounds*	*Pounds*	*Per cent*	*Per cent*
A	34.8	5.06	2.41	29.5	14.7
B	47.2	5.41	2.40	30.1	22.0
C	50.2	5.81	2.43	30.4	24.1
D	53.0	4.84	2.39	33.8	26.7
E	58.8	5.01	2.30	36.4	25.0
F	61.8	4.71	2.07	39.3	32.6
G	62.8	3.88	2.06	41.3	34.9
H	65.0	4.22	2.00	42.1	43.5
I	65.2	4.25	2.03	47.0	39.9
J	71.6	3.84	1.89	48.1	35.2
K	86.6	2.36	1.43	58.7	44.5
L	99.4	1.88	1.07	66.4	51.5
Check	99.6	2.17	1.18	68.7	48.1

green weight of fruit, green weight of vine less fruit, number of dead, sick, and healthy leaves per plant (5 plants only per plot). The data have been studied by graphic comparison.

Relation between Infection as Judged and as Counted

The relation is linear between the infection index obtained by the subjective judging technique and the percentage of leaves killed or the percentage of fruits invaded by stem-end rot, obtained by the more objective counting technique (Fig. 1, A and B). When the curves are extrapolated, they intersect the abscissa at the zero point in each case. It is clear that the judgment

method of recording magnitude of infection is not only valid and, therefore, sufficiently objective within this experiment, but it also is precise.

It is noteworthy that the curve relating killed leaves to infection index reaches the ceiling of infection index at about 65 per cent of killed leaves.

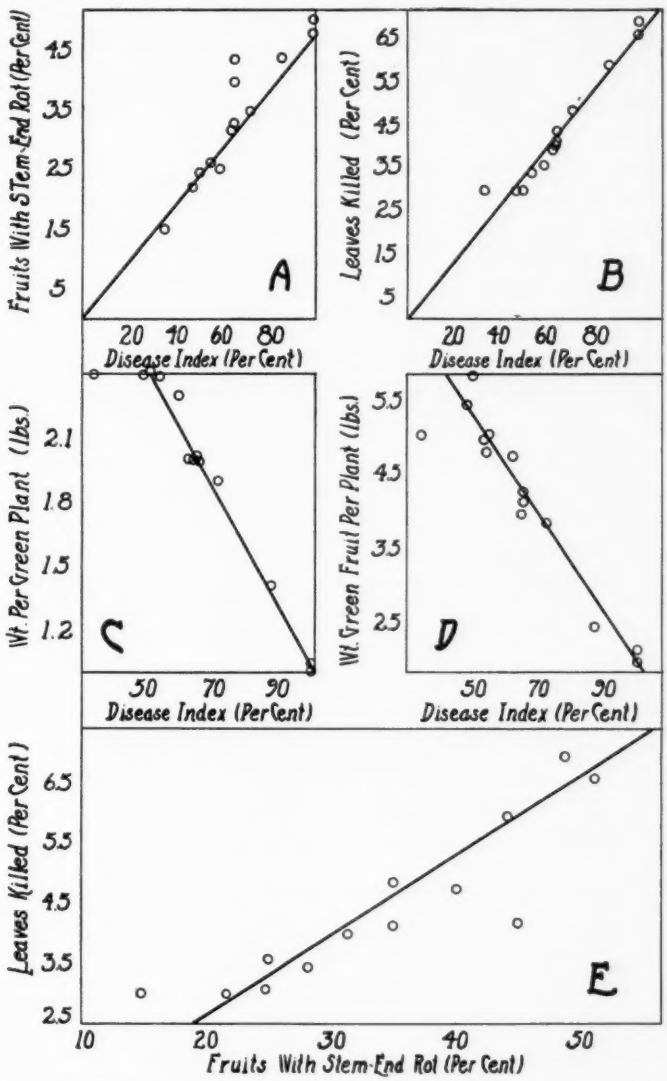

FIG. 1. A graphic comparison of methods of measuring magnitude of infection by *Alternaria solani* on tomatoes. A. Relation between infection index and percentage of fruits affected by stem-end rot. B. Relation between infection index and percentage of leaves killed. C. Relation between infection index and weight of green plants less fruit. D. Relation between infection index and weight of green fruits at the end of the season. E. Relation between percentage of fruits affected by stem-end rot and percentage of leaves killed.

The reason for this seems clear. The visual readings are based on the *area* of leaves, whereas the counting system is based on the *number* of leaves that

have been killed. In the counting system a relatively resistant tiny new leaf, just unfolded at the apex of the stem, carries as much weight as a big susceptible leaf at the base, so that not more than 65 per cent of the total number of leaves may have been killed on plants that look almost denuded of effective foliage. Stated otherwise these data emphasize clearly that counting the number of leaves killed does not give as true a picture of the number of successes registered by the fungus as an estimate of the leaf area destroyed, despite the fact that the former is a more nearly objective method than the latter.

Since both the percentage of infection on leaves and percentage of infection on fruit bear a linear relation to infection index, it follows that the relation between the two should be linear. A simple plotting of the data (Fig. 1, E) shows that such is the case.

Relation between Infection Index and Weight of Green Fruit and Green Plant

The weight of green fruit and the weight of the green plant less fruit at the end of the season can be measured objectively. They should be closely correlated inversely with the infection index because the tissues not directly affected by disease are present for weighing. It was of interest, therefore, to observe a linear relation between disease index and weight of green fruit and weight of green plants less fruits (Fig. 1, C and D). Here it is also clear that the grouping technique gives valid and precise data.

Agreement between Workers

During 1940 a similar experiment was made on Scarlet Dawn tomatoes at New Haven, Conn., comparing 7 copper materials with a check, on 4 replicate plots of 10 plants each, making a total of 280 plants. On September 7, when the disease was becoming serious, the plots were rated independently by each of 3 workers after a preliminary conference on the groupings. Data (Table 2) show that every material, with a single exception, was

TABLE 2.—*Readings of disease index on plots of tomatoes treated with different fungicides to control Alternaria solani*

Treatment	Reader No. 1	Reader No. 2	Reader No. 3
A	62.5	66.5	66.2
B	63.0	69.0	68.3
C	76.0	73.5	77.0
D	79.0	78.5	79.5
E	80.5	79.5	79.0
F	87.5	86.5	84.0
G	90.5	87.5	87.5
H	99.5	98.5	98.0

rated in precisely the same order by all 3 readers, even when the materials differed by only 2 or 3 per cent. The single exception is materials D and E, where D was rated $\frac{1}{2}$ per cent better and 1 per cent better than E by readers

No. 1 and No. 2 respectively, but it was rated ½ per cent poorer by reader No. 3.

Usefulness for Dosage-control Curves

There is one last bit of interesting evidence of the precision of the technique. Recently, it has been shown that dosage can be related in a linear fashion to disease control (1) and that data obtained with the McKinney (5) technique fit the curves smoothly.

DISCUSSION

The precision of the McKinney infection-index technique is most striking, as shown by the linear relations to the counted percentage defoliation, percentage of stem-end rot, green weight of vine less fruit, and green weight of fruit. The agreement for most of the points is so close that any point off the curve is worthy of further investigation as an aberrant case, not as throwing doubt upon the validity of the agreement. In the case of weight of green plant less fruit the only point off the curve is the first one. This shows lower green weight than expected. This point is for Bordeaux mixture, which is already known to dwarf tomatoes.

The technique is somewhat less than completely satisfactory for comparing one year with the next, because it is difficult to keep the standards unchanged. This would also be a difficulty in comparing records at one research station with those at another. Nevertheless, for one investigator and especially for one series of plots, the technique is well adapted.

It is probable, however, that any judgments are influenced by the general level reached in any particular season. If most of the plants are infected, a mediocre plant may pass for excellent, but if most plants are not infected a mediocre plant may pass for poor.

This difficulty can be reduced by preparing charts as standards, such as those used by the cereal-rust investigators.

SUMMARY

The McKinney rapid technique for measuring the magnitude of infection of a plant disease has been applied to tomato defoliation.

Each plant is examined by walking crosswise of the plots and assigned an arbitrary number from 0 to 4, ranging from no disease to complete defoliation. The ratings are summed, divided by the total number of plants multiplied by 4, since 4 is the highest possible amount of disease. The quotient is multiplied by 100 to convert to percentage, which is called index of infection.

This rapid, if somewhat subjective, method of measuring infection was found to bear a linear relation to the following approximately objective methods of reading infection on the same plots: (1) percentage of leaves killed by the fungus, (2) percentage of fruits invaded by stem-end rot, (3) weight of green plants less fruits at time of reading, and (4) weight of green

fruits at the end of the season. Using this rapid technique 3 men read 8 spray treatments and rated them in the same order of effectiveness.

From these facts it follows that the method is valid, precise, and sufficiently objective.

Although the method is precise for reading a single series of fields or plots, it has the loophole that the standards are difficult to maintain precisely from season to season.

LITERATURE CITED

1. DIMOND, ALBERT E., J. G. HORSFALL, J. W. HEUBERGER, and E. M. STODDARD. Role of the dosage-response curve in the evaluation of fungicides. Conn. Agr. Exp. Stat. Bull. 451. 1941.
2. HORSFALL, JAMES G., and J. W. HEUBERGER. Causes, effects and control of defoliation on tomatos. Conn. Agr. Exp. Stat. Bull. 455. In press. 1941.
3. MARSH, R. W., H. MARTIN, and R. G. MUNSON. Studies upon the copper fungicides. III. The distribution of fungicidal properties among certain copper compounds. Ann. Appl. Biol. **24**: 853–866. 1937.
4. MARTIN, W. H. Studies on tomato leaf spot control. New Jersey Agr. Exp. Stat. Bull. 345. 1920.
5. MCKINNEY, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Jour. Agr. Res. [U.S.] **26**: 195–217. 1923.
6. STEVENS, NEIL. Botanical research by unfashionable technics. Science **93**: 172–176. 1941.

ISOLATION AND INFECTION TESTS WITH SEED- AND SOIL-BORNE COTTON PATHOGENS[1]

W. W. Ray and J. H. McLaughlin

(Accepted for publication July 7, 1941)

Seedling diseases of cotton are important to the grower because of poor stands, the necessity of replanting, stunted plants, decrease in production because of skips in rows, excess amount of seed required, the many seedling diseases that later develop into lint- and boll-destroying diseases, the lessened value and amount of lint cotton produced per unit area, and the weakening of plants, making it much easier for secondary organisms to infect the cotton.

Damping-off and other seedling diseases of cotton plants may be caused and accelerated by a number of organisms. Arndt (1) isolated the following organisms from diseased seedlings: *Glomerella gossypii, Fusarium* spp., *Rhizoctonia solani, Pythium ultimum,* and several species of bacteria. Of these organisms, *G. gossypii* caused the most damping-off, whereas the various species of *Fusarium* produced lesions on roots and hypocotyls, but not damping-off. *Rhizoctonia solani,* isolated in but 10 per cent of the attempts, was not considered important as a cause of damping-off in cotton. Lehman (4) tested 35 seed lots of the 1936 cotton crop from 19 counties in North Carolina. He found 40 per cent of the seed in 31 of the lots infested with *G. gossypii* and 31.4 per cent of the seed of all lots infested with various species of *Fusarium* among which *F. moniliforme* was frequently recognized. Camp *et al.* (2) report from a survey in Florida the diseases most common during the first two months of the season to be sore shin, caused by several organisms, particularly *Rhizoctonia solani;* angular leaf spot (*Bacterium malvaccarum*); and wilt (*Fusarium vasinfectum*). They also reported the presence of a large proportion of *F. moniliforme.*

Miller and Weindling (6, 7) and Weindling *et al.* (8) have shown that *Glomerella gossypii* is rarely found in Oklahoma and Texas, but that it is the fungus most often isolated from diseased seedlings in the Southeastern States. Recent surveys of our own substantiate their data with reference to the scarcity of *G. gossypii* in Oklahoma, for it has been found in but few counties in the eastern part of the State.

In many respects the organisms isolated from diseased seedlings in Oklahoma are similar to those reported by Miller and Weindling in the past few years for the other cotton-belt States.

Weindling *et al* (8) have shown that *Fusarium moniliforme* is more often associated with diseased seedlings than is any other fungus in Oklahoma. Our data have shown this to be true. Although it has been shown by Wood-

[1] Published with the approval of the Director of the Oklahoma Agricultural Experiment Station.

Acknowledgments are made to Dr. K. S. Chester for helpful suggestions on the experiments and preparation of the manuscript and to Dr. C. D. Sherbakoff for his aid in the identification of the Fusaria.

roof (9) that this fungus is pathogenic, our tests have shown that it is not so severe in its attack as *G. gossypii*, *Rhizoctonia solani*, and a few other species of *Fusarium*. But because of its great frequency in Oklahoma soils, this fungus must be considered an important cause of seedling diseases of cotton.

Rhizoctonia solani is the second most commonly isolated fungus from seedlings in Oklahoma. There is no doubt about its importance as a cotton pathogen.

Since *Glomerella gossypii* is of minor importance in Oklahoma, a condition contrary to that east of this State, an attempt has been made to classify the organisms causing seedling diseases on the basis of their relative importance in this State. Also it was thought necessary to identify the various species of *Fusarium* and to establish their importance, if any, as pathogens. To accomplish these aims, it was first our purpose to collect samples of diseased seedlings throughout Oklahoma and to isolate and identify the organisms present. Samples have been taken for a period of 2 years, and more will be taken in the future. A second purpose of this study was to test the pathogenicity of the organisms (Table 2) under various environmental conditions and to rank the organisms on the basis of these tests according to their importance as cotton pathogens.

TABLE 1.—*Tabulation of the more pathogenic fungi isolated from cotton seedlings in 21 counties of Oklahoma in 1939*

Name of the organism	No. of times isolated	Percentage of fungi isolated
Fusarium moniliforme	61	40.00
F. scirpi	8	3.90
F. vasinfectum	5	2.40
F. scirpi v. *acuminatum*	2	0.90
F. solani	21	10.30
F. solani v. *martii*	20	9.90
F. equiseti v. *bullatum*	1	0.49
Rhizoctonia solani	40	19.80
Sclerotium bataticola	24	11.80

FUNGI ASSOCIATED WITH DISEASED COTTON SEEDLINGS

In table 1 is presented a list of the more pathogenic fungi with the percentage of times they were isolated from field samples taken in 21 counties of Oklahoma in 1939. This list does not include such commonly isolated genera as *Chaetomium*, *Rhizopus*, *Aspergillus*, *Alternaria*, *Penicillium*, and some sterile forms, for tests have shown that such fungi are either saprophytes or weak parasites.

FUNGI ASSOCIATED WITH COTTON SEEDS AND BOLLS

Numerous isolations of fungi have been made from seed, particularly from their interiors, and fewer have been made from boll lesions. A tabulation of the organisms isolated from seedlings, seeds, and bolls is given in table 2.

TABLE 2.—*A list of fungi isolated from seedlings, seeds, and bolls of the cotton plant*

Name of the organism	Isolated from		
	Seedlings	Seeds	Bolls
Glomerella gossypii (South.) Edg.	+	+	+
Fusarium moniliforme Sheld.	+	+	+
F. vasinfectum Atk.	+	−	−
F. scirpi Lamb and Fautr.	+	+	+
F. solani (Mart.) App. and Wr.	+	+	+
F. semitectum Berk. and Rav.	−	+	+
F. chlamydosporum Wr. and Rg.	−	+	−
F. scirpi L. and F. v. *acuminatum* (E. and E.) Wr.	+	+	+
F. equiseti (Cda.) Sacc. v. *bullatum* (Sherb.) Wr.	+	+	−
Rhizoctonia solani Kühn	+	−	−
Sclerotium bataticola Taub.	+	−	−
Alternaria spp.	+	+	+
Penicillium spp.	+	−	+
Aspergillus spp.	+	−	+

PATHOGENICITY TESTS OF THE MORE IMPORTANT COTTON FUNGI

Materials and Methods

The cotton seed used in these experiments consisted of 3 varieties, namely, Acala, from the 1938 Oklahoma crop, Paymaster, from the 1939 Texas crop, and D. and P. L. 11A, from the Mississippi crop of 1938.

Experiments were conducted to test the pathogenicity of the various organisms by: (a) inoculation of the organisms into sterile soil and subsequent planting of sterile cotton seed, and (b) inoculating cotton seedlings grown under sterile conditions in test tubes on water agar.

The seeds were delinted with concentrated sulphuric acid and graded as suggested by Chester (3), and only the heavy, fungus-free fraction was used. These were then surface-sterilized by immersing in a calcium hypochlorite solution containing 2.79 per cent of available chlorine.

Five cultures of each organism were grown on agar slants, and at the end of 10 days the contents of the tubes were removed and ground in a small amount of sterile sand. This was then added to enough sterilized soil[2] to fill twenty $2\frac{1}{4}$-in. pots. Three seeds were planted in each pot and kept moist with sterile water. Data were obtained at the end of 28 days in most tests.

It was not possible to control the environmental factors within definite, small limits, but the environmental differences between the various experiments were diverse enough to give a distinct contrast. Rather than tabulate the lengthy data of these experiments, which are somewhat preliminary in nature, only a summary will be given with special reference to those organisms possessing the highest degrees of virulence.

EXPERIMENTS

1. *Standard Conditions.* With the average temperature of the greenhouse at 80° F. and with ample water for the plants, the 4 most virulent

[2] The soil for these experiments consisted of 2 parts loam, 1 part sand, and 1 part sewer sludge.

organisms of those tested were, in order: *Glomerella gossypii, Rhizoctonia solani, Fusarium scirpi* and *F. moniliforme*. Some of the other fungi produced minor lesions on the roots and hypocotyl, but no serious damage was done to the seedlings.

2. *Excessive Moisture.* When the plants were watered heavily several times a day in the 80-degree greenhouse, all of the organisms tested showed an increase in virulence with the exception of *Fusarium vasinfectum*, which decreased. *F. moniliforme* displayed a decided increase in pathogenicity.

3. *Deficient Moisture.* The soil was watered just enough to keep the plants growing. *Glomerella gossypii, Rhizoctonia solani,* and *Fusarium scirpi* were highly pathogenic, whereas the other fungi tested decreased in their virulence.

4. *Low Temperature.* The plants were held for several days at 80° F. and then placed outdoors, where the temperature averaged near 65° F. Both *Glomerella gossypii* and *Rhizoctonia solani* were very pathogenic and caused damping-off. The other organisms in the test did no serious injury to the plants.

5. *Alkaline Soil.* Enough lime was added to the soil to give a reaction of pH 8.3. *Glomerella gossypii* and *Rhizoctonia solani* were the only fungi of those tested that were virulent, and they caused considerable damping-off. The other fungi did no noticeable damage to the plants.

6. *Acid Soil.* Enough sulphur was added to the soil to give a reaction of pH 6.3. *Rhizoctonia solani,* followed closely by *Glomerella gossypii,* was the most pathogenic of the organisms tested. The remainder of the fungi produced but slight infections with no prominent injury to the plants.

DISCUSSION

Since the results of these various experiments are not directly comparable, it seemed desirable to arrange a classification of the organisms involved according to their relative degrees of pathogenicity. In these tests 3 degrees of injury to the plants were recognized: *slight,* no evidence of injury observable, yet root systems visibly attacked; *moderate,* root systems obviously attacked but with only slight injurious effect upon the plants; and *heavy,* root systems and plants displayed serious injury or death. Arbitrarily, heavy infection was given a value of 3 points, moderate infection 2 points, and slight infection 1 point. An index of infection for each organism in each experiment was calculated by multiplying the percentage of plants heavily infected by 3, moderately infected by 2, and slightly infected by 1, totaling, and averaging.

Since *Glomerella gossypii* proved in these experiments to have the highest index of infection, it was given an arbitrary weighted index of 100. The indices of infection of all the other fungi were weighted in proportion to the arbitrary weighted index of infection assigned to *G. gossypii.* The weighted indices of the organisms employed in the various experiments is indicated in table 3.

TABLE 3.—*The weighted indices of infection of the fungi used in the soil culture experiments. The letters and numbers refer to the identity of the isolate used in the experiments*

Name of the organism	W. I.	Name of the organism	W. I.
Glomerella gossypii	100	*Sclerotium bataticola* (41)	45
Rhizoctonia solani	86	*Fusarium semitectum*	42
Fusarium scirpi (D)	58	*F. moniliforme* (115)	40
F. scirpi v. *acuminatum*	56	*F. vasinfectum* (24)	37
F. chlamydosporum	53	*F. moniliforme* (18)	35
F. moniliforme (110)	52	*F. vasinfectum* (27)	34
F. vasinfectum (27)	48	*Sclerotium bataticola* (R37)	30
F. scirpi (44A)	47	*Aspergillus* sp.	29
F. solani	46	*Penicillium* sp.	28
F. equiseti v. *bullatum*	46		

The pathogenicity of the various fungi was determined also by the inoculation of seedlings grown in large test tubes on water agar. Using the "weighted index of infection" method, and applying it to the test tube experiments, a definite correlation with the results obtained in the pot cultures was established. In general, it was found that the weighted indices were higher than for the pot cultures, but in the same relative order.

Although field tests have not been attempted, it is predicted that the infection indices will be lower due to a lower inoculum potential and competition from other soil organisms.

CONCLUSIONS

Glomerella gossypii, despite its high degree of pathogenicity, is probably of little importance in Oklahoma because of its infrequent occurrence.

Apparently strains varying in pathogenicity exist in *Fusarium moniliforme*, *F. vasinfectum*, *F. scirpi*, and *Sclerotium bataticola*, as evidenced by the weighted indices of infection of the several isolates of these species.

Sclerotium bataticola (*Rhizoctonia bataticola* (Taub.) Butler), although common in Oklahoma soils, is of slight consequence as a pathogen of cotton seedlings.

Fusarium moniliforme, although it does not possess a high degree of virulence, might conceivably become, because of its high frequency of occurrence, a serious cotton-seedling parasite under favorable conditions.

The majority of the other species of *Fusarium* and those of *Alternaria*, *Penicillium*, *Chaetomium*, *Aspergillus*, and *Rhizopus* are not considered important parasites of cotton seedlings in Oklahoma, either because of their lack of virulence or because of their scarcity in the soil.

Rhizoctonia solani, because of its high degree of virulence under various environmental conditions, and because of its frequency in Oklahoma soils, is considered to be the most important fungus involved in diseases of cotton seedlings. The real importance of this fungus may not be indicated by the actual percentage of isolation for several reasons. Tests in our greenhouse and those by Lehman (5) show that seedlings cannot be protected satisfactorily against this organism by the seed treatments thus far employed. We

have observed here that Ceresan-dusted seed, planted in soil heavily infested with this fungus, often do not emerge at all. It is conceivable that the long skips in rows so commonly observed in the fields throughout the State may be due largely to a high inoculum potential in the soil. In such a situation no samples from which to make isolations are taken.

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LITERATURE CITED

1. ARNDT, C. H. The etiology of damping-off of cotton seedlings. Phytopath. **25**: 968–969. 1935.
2. CAMP, A. F., *et al.*, Plant diseases. Fla. Agr. Exp. Stat. Rept. (**1928**): 38–39. 1928.
3. CHESTER, K. S. Gravity grading, a method for reducing seed-borne diseases in cotton. Phytopath. **28**: 745–749. 1938.
4. LEHMAN, S. G. Seed infestation with *Glomerella* and *Fusarium* in the 1938 cotton crop of North Carolina. U. S. Dept. Agr., Bur. Pl. Ind., Pl. Dis. Rptr. **22**: 4–6. 1938.
5. ———. Cotton seed dusting in relation to control of seedling infection by *Rhizoctonia* in the soil. Phytopath. **30**: 847–853. 1940.
6. MILLER, P. R., and R. WEINDLING. A survey of the cotton seedling diseases in 1939 and the fungi associated with them. U. S. Dept. Agr., Bur. Pl. Ind., Pl. Dis. Rptr. **23**: 210–214. 1939.
7. ———, and ———. A survey of cotton seedling diseases in 1940 and the fungi associated with them. U. S. Dept. Agr., Bur. Pl. Ind., Pl. Dis. Rptr. **24**: 260–263. 1940.
8. WEINDLING, R., P. R. MILLER, and A. J. ULLSTRUP. Fungi associated with diseases of cotton seedlings and bolls, with special consideration of *Glomerella gossypii*. Phytopath. **31**: 158–167. 1941.
9. WOODROOF, N. C. A disease of cotton roots produced by *Fusarium moniliforme* Sheld. Phytopath. **17**: 227–237. 1927.

A PHYSIOLOGICAL STUDY OF THE SUSCEPTIBILITY OF THE BLUSHED AND GREEN SIDES OF APPLES TO CERTAIN FUNGOUS ROTS[1]

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INTRODUCTION

As the season advances, most apple fruits gradually develop a blush on the side facing the sun. Brooks and Fisher (1926) found that the osmotic concentration of the apple sap was higher on the blushed side than on the green side. Haynes and Archbold (5) report higher sugar content on the blushed side of maturing apple fruits. Other internal differences also were thought to exist. The present study was undertaken to test the difference in susceptibility of the blushed and green sides of apple to certain fungous rots and its relation to certain physical and chemical properties of the two sides.

MATERIALS AND METHODS

Two tests were made on July 22 and August 1, 1940. In the first test 4 varieties of apple, Wealthy, McIntosh, Cortland, and Snow, were inoculated with 4 fungi, namely, *Sclerotinia fructicola* (Wint.) Rehm., *Penicillium expansum* Link, *Physalospora malorum* (Peck) Shear, and *Lambertella corni-maris* von Höhnel. In the second test one other variety of apple, Duchess, was added but only 3 of the organisms were employed, *P. malorum* being omitted. Along with the inoculation tests internal factors such as water content, total nitrogen content, total acidity, sugar content, and tissue firmness were determined.

Inoculation. Inoculations were made on the blushed and green sides of 16 fruits of each variety of apple with each fungus. A pyramidal-shape block of tissue was cut out from the side of each apple and into the cavity thus formed a small rectangular piece of mycelium, cut out from the margin of an actively growing Petri-dish culture, was inserted. The block of tissue was then replaced without sealing. Care was used in cutting the blocks of apple tissue and the pieces of mycelium for inocula to ensure uniformity in all inoculations. The inoculated apples were put in paper bags and kept in moist chambers consisting of wooden apple boxes lined with pliofilm and equipped with tight covers.

Measurement. The diameter of the rotted area of the fruit was measured 3 days after inoculation with *Sclerotinia fructicola,* 5 days after inoculation with *Penicillium expansum,* 6 days after inoculation with *Physalospora*

[1] This paper is one section of a thesis submitted to the faculty of the graduate school of Cornell University for the degree of Doctor of Philosophy.

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malorum, and 10 days after inoculation with *Lambertella corni-maris*. The method of measurement consisted in slicing off a portion of the apple tissue at the bottom of the inoculation cavity through the line XY, as shown in figure 1, A, and measuring the diameter (the distance CD, Fig. 1, A) of the

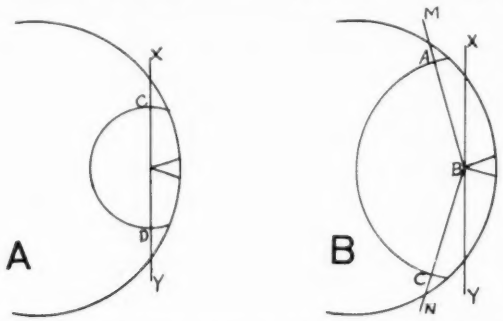

FIG. 1. A and B illustrate points made in the text concerning methods of measuring the rotted area of apple fruits.

rotted area thus exposed. In case the decay was too far advanced, as is shown in figure 1, B, a slice of the tissue was first cut off through the line XY, and then another slice was cut through the lines BM and BN, respectively, on either side. The sum of the lines AB and BC was taken as the diameter of the rotted area.

Determination of the Internal Factors. Apples for such determinations were picked from the same trees as those from which fruits were picked for inoculation. Duplicate composite samples were prepared from 20 apples of each variety for the determination of water content, nitrogen content, total acidity, and sugar content.

For the determination of water content, the apple was pared, and 3 longitudinal slices of approximately equal size were cut from the fruit and placed in a Petri dish that had been lined with filter paper and weighed. The Petri dish was kept covered so that an excessive loss of moisture from the sample might be avoided. The sample was quickly weighed, the total fresh weight of each sample being 35 to 42 grams. It was then dried in a ventilated Freas electric oven at 60° C. for about 72 hours and again weighed.

The dried sample, together with the filter paper from the moisture determination, was digested, and the total nitrogen content was determined by Gunning's modification of the Kjeldahl method.

For the acidity determination the apples were likewise pared, and several longitudinal slices were cut from each of the 20 apples and placed in a wide-mouth bottle. The composite sample was frozen and thawed. The juice was pressed out with a metal hand press and centrifuged for 4 minutes. A 10-cc. sample of the supernatant liquid was pipetted out and placed in a 150-cc. Erlenmeyer flask. About 20 cc. of distilled water was added. The juice was titrated against N/10 NaOH solution using phenolphthalein as an indicator. A satisfactory absolute end-point was difficult to get, but a comparative end-point was easily obtained when the pinkish shadow of the dark-

colored juice was cast upon a white table and compared with that of the first sample, as a lamp shone through the two samples from an equal distance of about 8 inches.

For the determination of sugar content the fruit was pared, and a small tangential section was sliced off, and then small cubes about 1 mm. across were cut and placed directly in a 150-cc. beaker kept in a moist chamber. The composite sample, averaging about 40 g., was quickly weighed and dumped into 50 cc. of boiling, 95 per cent alcohol in a wide-mouth bottle. Heating was continued for 15 minutes so as to stop enzyme action. The sample was later extracted in a Soxhlet extractor, with about 100 cc. more of 95 per cent alcohol, for 5 hours. The extract was evaporated down to 10 cc. 3 times and then diluted with 20 cc. of warm distilled water, cleared, deleaded, made up to volume, and analyzed for reducing and total sugars by the official gravimetric method.

For the determination of tissue firmness, 20 apples of each variety were tested with a Magness pressure tester. A small portion of the skin was sliced off, and the pressure tester was then applied to the exposed cut surface of the flesh. The firmness was measured by the pounds of pressure required to puncture the tissue.

RESULTS

The results of the tests are presented in table 1 and discussed below.

Difference in Susceptibility of the Two Sides of Apple

Sclerotinia fructicola. With this fungus the rate of rotting was invariably faster on the green than on the blushed side, and the difference was statistically significant in 7 out of 9 cases. When all the data of both inoculation tests were analyzed together, the odds were found to be over 9999 : 1, the decay being more rapid on the green side.

Penicillium expansum. In the first inoculation with *P. expansum* the green side of 3 out of 4 varieties of apple rotted faster than the blushed side, but only in the variety Wealthy is the difference statistically significant. In the second inoculation, the fungus invaded 4 of the 5 varieties significantly faster on the green than on the blushed side; in the other variety the rate of rotting was about equal on the two sides. Analysis of the data of the two inoculations together showed significantly more rapid rotting of the green side.

Physalospora malorum. The rate of rotting on the blushed side of two apple varieties was faster than that on the green side in the test with this fungus; in the other two varieties the order was reversed, and the difference was small. When the data on the 4 varieties were analyzed together, the difference was insignificant.

Lambertella corni-maris. Analysis of the data as a whole showed no significant difference between the two sides in rate of rotting by this fungus.

TABLE 1.—*The relation of the rate of rotting of apple to certain physical and chemical properties of the blushed and green sides of the fruit*

Date	Variety	Side	Diameter of rotted area (mm.)				Water (per cent fresh weight)	Nitrogen (per cent fresh weight)	Total acidity[b]	Sugar (per cent fresh weight) Total reducing sucrose			Tissue firmness (pounds pressure to puncture)
			Physalospora malorum (6 days)	*Lambertella corni-maris* (10 days)	*Penicillium expansum* (5 days)	*Sclerotinia fructicola* (3 days)							
Aug. 22	Wealthy	Blushed	18.1[a]	25.1	18.5[a]	22.3[a]	88.2	0.029	13.64	.....	.....	.....	24.2[a]
		Green	16.1	24.8	20.4	24.4	90.3	0.025	12.84	.....	.....	.....	21.9
	McIntosh	Blushed	15.9	24.4	12.7	30.1[a]	85.5	0.037	9.84	.....	.....	.....	23.0[a]
		Green	16.4	24.8	12.9	33.1	87.5	0.034	10.18	.....	.....	.....	20.5
	Cortland	Blushed	16.6[a]	24.1[a]	14.1	26.0[a]	85.5	0.047	7.17	.....	.....	.....	23.7[a]
		Green	14.7	22.8	15.1	28.4	87.3	0.042	6.55	.....	.....	.....	22.1
	Snow	Blushed	16.7	26.8[a]	16.9	22.1[a]	85.6	0.027	11.61	.....	.....	.....	28.4[a]
		Green	17.4	28.2	16.6	23.8	86.5	0.026	11.33	.....	.....	.....	26.6
Sept. 1	Wealthy	Blushed	.....	12.5	22.4	25.3[a]	88.2	0.028	11.88	8.59	7.58	1.01	18.1[a]
		Green	.....	12.9	22.3	26.9	89.7	0.027	11.75	7.13	6.42	0.71	17.2
	McIntosh	Blushed	.....	13.2	18.2[a]	21.2[a]	85.1	0.029	13.58	9.71	8.21	1.50	22.3[a]
		Green	.....	13.2	19.3	23.4	86.9	0.029	13.64	8.39	7.58	0.81	20.0
	Cortland	Blushed	.....	17.6	18.3[a]	26.2[a]	85.3	0.041	7.39	9.42	7.87	1.55	23.1[a]
		Green	.....	17.1	19.9	28.3	87.0	0.042	6.69	8.55	6.88	1.67	22.1
	Snow	Blushed	.....	19.8	19.9[a]	24.5	85.6	0.026	11.47	8.60	7.20	1.40	26.2[a]
		Green	.....	19.1	20.6	24.8	86.5	0.026	12.13	7.97	6.69	1.28	25.0
	Duchess	Blushed	.....	17.6	27.1[a]	28.3	89.1	0.032	13.96	8.24	7.02	1.22	14.2[a]
		Green	.....	15.7	28.3	29.6	89.8	0.030	13.92	7.49	6.22	1.27	13.1

[a] indicates that the difference is statistically significant.
[b] cc. N/10 NaOH to neutralize 10 cc. juice.

Difference in Physical and Chemical Properties of the Two Sides of Apple

Water Content. In both determinations the water content was slightly but consistently higher on the green than on the blushed side of all varieties tested.

Nitrogen Content. The nitrogen content was consistently higher on the blushed side in the first determination, but there were 2 ties and 1 reverse out of 5 in the second determination. Analysis of the data of both determinations together shows that the nitrogen content of the blushed side was significantly higher than that of the green side. The differences all were very small.

Total Acidity. There was no consistent difference in the total acid content of the two sides of the varieties tested.

Sugar Content. Total and reducing sugars were consistently higher on the blushed side, but there was no consistent difference in the sucrose content of the two sides of the apple fruit.

Tissue Firmness. The blushed side of the fruit was invariably firmer than the green side. The difference was highly significant in all cases.

DISCUSSION

The blushed side of apples is firmer and has higher contents of sugars and total nitrogen and lower water content than the green side; the difference in the acid content of the two sides is inconsistent. The results on the water, sugar, and nitrogen contents are in agreement with those reported by Brooks and Fisher (3) and by Haynes and Archbold (5).

The slower rate of rotting of the blushed side of apples by *Penicillium expansum* and *Sclerotinia fructicola* is correlated with the higher contents of sugars and nitrogen, the lower content of water, and the greater firmness of tissue. If the amount of available food determined the rate of invasion by these two fungi, the blushed side of the apple, containing more sugar and nitrogen, would be expected to rot faster; however, the reverse has been found to be true. It is hardly likely that any of the chemical constituents of the fruit would be so concentrated on the blushed side as to become toxic to the fungi. Nor is it conceivable that a difference of 1 or 2 per cent in the water content of the two sides would cause a change in their susceptibility. Thus is would seem that among the factors tested, a possible explanation for the difference in susceptibility of the two sides of the apple to these two rots lies in their difference in tissue firmness. In the case of *Physalospora malorum* and *Lambertella corni-maris*, however, there was no consistent difference in the rate of rotting of the two sides of the apple fruit. This indicates that the penetration of the fruit tissue by these two fungi was unaffected by the internal factors determined.

In a study of toxic and enzymatic activities of juice expressed from rotted apples, the writer[3] found that the juice of apples rotted by

[3] Unpublished material.

Penicillium expansum or *Sclerotinia fructicola* contained a considerable amount of protopectinase which macerated thin discs of apple, potato, and carrot while the juice of apples rotted by *Physalospora malorum* or *Lambertella corni-maris* possessed little or no macerating property. On the other hand, juice from apples rotted by *P. malorum* and *L. corni-maris* was found to possess a distinct toxic property indicated by the discoloration of the tissue discs, while the juice from apple rotted by *P. expansum* or *Sclerotinia fructicola* showed no noticeable toxic action. This is in line with the work of Behrens (2), who, in experiments with a *Penicillium* species, shows that the fungus is intercellular and secretes an active middle-lamella-dissolving enzyme, and of Valleau (9), who, in a study of brown rot of plum caused by *S. fructicola*, also demonstrates that the fungus is intercellular and believes that it penetrates through the middle lamella of the suscept cell wall by dissolving the pectic substance with an enzyme.

It has been shown repeatedly that the firmness of fruit tissue is correlated, to a large extent, with the amount of insoluble pectic compounds in the cell wall (Magness and Diehl (6), Appleman and Conrad (1), Plagge *et al.* (7), Haller (4), Smock and Allen (8), and Van Doren (10). Such being the case, the greater tissue firmness of the blushed side would suggest that this side contains more insoluble pectic substance and offers greater resistance to penetration by *Penicillium expansum* and *Sclerotinia fructicola* which are intercellular and make their advance by secreting a middle-lamella-dissolving enzyme, protopectinase. The tissue firmness or the amount of insoluble pectic substance, however, has no effect on the rate of advance of the other fungi, *Physalospora malorum* and *Lambertella corni-maris*, which are presumably intracellular and kill suscept cells ahead of penetration by means of one or more toxic substances.

SUMMARY

It was found that *Penicillium expansum* and *Sclerotinia fructicola* rotted the blushed side of apple fruit more slowly than they did the green side while *Physalospora malorum* and *Lambertella corni-maris* showed no difference in their rate of rotting of the two sides.

The blushed side of apple fruit was found to be firmer and to contain higher contents of sugars and nitrogen and lower content of moisture. The acid content of the two sides did not show any consistent difference.

The slower rate of rotting of the blushed side of apple by *P. expansum* and *S. fructicola* is correlated with higher contents of sugars and nitrogen, lower content of water, and greater firmness of tissue.

As an explanation to account for the difference in the rate of rotting of the two sides of apple by *P. expansum* and *S. fructicola*, it is suggested that the blushed side being firmer possibly contains more insoluble pectic substance and is thus more resistant to the invasion by these organisms which are supposed to be intercellular and penetrate through the cell wall of the suscept by means of enzyme action.

LITERATURE CITED

1. APPLEMAN, C. O., and C. M. CONRAD. Pectic constituents of peaches and their relation to softening of the fruit. Maryland Agr. Exp. Stat. Bull. 283. 1926.
2. BEHRENS, J. Beiträge zur Kenntnis der Obstfäulnis. Centralbl. Bakt. 2: 514–522, 547–553, 635–644. 1898.
3. BROOKS, C., and D. F. FISHER. Some high-temperature effects in apples: Contrasts in the two sides of an apple. Jour. Agr. Res. [U.S.] 32: 1–16. 1926.
4. HALLER, M. H. Changes in the pectic constituents of apples in relation to softening. Jour. Agr. Res. [U.S.] 39: 739–746. 1929.
5. HAYNES, D., and H. K. ARCHBOLD. Chemical work on fruit. Dept. Sci. Ind. Res. (London), Food Invest. Board Rept. (1933): 225–228. 1934.
6. MAGNESS, J. R., and H. C. DIEHL. Physiological studies on apples in storage. Jour. Agr. Res. [U.S.] 27: 1–38. 1924.
7. PLAGGE, H. H. *et al.* Certain physical and chemical changes of Grimes apples during ripening and storage period. Iowa Agr. Exp. Stat. Research Bull. 91: 42–72. 1926.
8. SMOCK, R. M., and F. W. ALLEN. Soluble pectin changes in gas-stored fruit. Amer. Soc. Hort. Sci. Proc. 35: 184–187. 1938.
9. VALLEAU, W. D. Varietal resistance of plum to brown rot. Jour. Agr. Res. [U.S.] 5: 365–395. 1915.
10. VAN DOREN, A. Physiological studies with McIntosh apples in modified atmosphere cold storage. Amer. Soc. Hort. Soc. Proc. 37: 453–458 (1939). 1940.

PHYSIOLOGICAL RESISTANCE TO HALO BLIGHT IN BEANS[1]

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During the past 8 years greenhouse and field tests on resistance to halo blight have been carried out with many bean varieties including a number of varieties reported resistant by other investigators. The severity of infection varied with varieties and, although certain varieties tended to escape infection in field tests, it was found that in many cases reportedly resistant varieties proved to be susceptible when inoculated in the greenhouse. However, in the case of several varieties reported resistant by Stapp,[2] together with a few other varieties, the occurrence of small, inconspicuous, brown, circular necrotic spots on inoculated leaves and the absence of typical halo lesions suggested that such varieties may become infected with halo blight without the production of typical halo lesions or serious damage to the plant. Furthermore, no evidence of systemic infection was ever observed in these varieties, although in parallel inoculations other varieties frequently showed veinclearing, stunting, wilting, or other symptoms of systemic infection with halo blight.

These observations strongly suggested the occurrence of physiological resistance to halo blight in certain varieties. Since the definite demonstration of this reaction would be of much value in further understanding the disease and of paramount importance in breeding for resistance to halo blight, experiments were set up to study further the behavior of varieties in which small, necrotic lesions instead of large, chlorotic lesions developed.

Two of these varieties were selected for a more detailed study of their reaction to halo blight infection under various conditions and with different types of inoculation in comparison with 2 susceptible varieties. This paper describes the results of these tests and presents a preliminary report on the behavior of progenies from certain crosses involving these resistant varieties.

MATERIALS AND METHODS

The two varieties selected for the study of their resistance were Red Mexican, a red-seeded, dry-land field bean, and Schwert, Hamburger Markt[3] (Stapp 27, F.P.I. 112,769). The two susceptible varieties used for comparison were Red Kidney and Bountiful.

Bean plants were grown singly in 5-in. clay pots in composted soil in the greenhouse at temperatures varying from 22–24° in the day time and 13–16° C. at night.

[1] Published with the approval of the Director as Paper No. 293, Journal Series, Nebraska Agricultural Experiment Station.

[2] Stapp, C. Fortgeführte Untersuchungen über die Resistenzverschiedenheiten von Bohnen (*Phascolus vulgaris*) gegen *Pseudomonas medicaginis* var. *phaseolicola* Burkh. Angew. Bot. **17**: 23–42. 1935.

[3] This variety, which will be referred to in this paper as Schwert No. 27, was obtained along with other varieties from Berlin, Germany, in 1935, and the number given each variety is that used by Stapp in his publication, see footnote 2.

The following inoculation methods were used: (a) Leaf and pod inoculations were made by spraying with a bacterial suspension in water. Previous to inoculation, plants were held in an incubation chamber at 24° C. with high humidity for 16 hours. After inoculation the plants were held in the incubator for 6 or 7 hours before removal to a greenhouse bench. In all cases not specified otherwise, plants were leaf-inoculated just as the third trifoliate leaves were unfolding, and pod inoculations were made during pod development. (b) Stem inoculations were made by stabbing twice through a smear of bacteria placed on the bean stems about $\frac{1}{4}$ in. below the primary leaves. (c) The method used in making germinated seed inoculations was a modification of one described by Stapp[4] and consisted of soaking germinated seeds for 4 hours in a dilute bacterial suspension in water. Seeds were germinated by placing them in a moistened rag-doll germinator held for 60 hours at 27–28°.

Isolates of *Phytomonas medicaginis* var. *phaseolicola* Burkholder, used in these studies, were isolates that, over a period of several years, had continuously produced typical halo-blight symptoms on Red Kidney, Giant Stringless Greenpod, Bountiful, and many other varieties. In all leaf and pod inoculations carried out for the purpose of varietal comparison all 4 varieties in any given test were inoculated with the same bacterial suspension. Forty-eight-hour-old cultures were used in all inoculation tests.

EXPERIMENTAL RESULTS

Four different inoculations were used in the comparative study of the reactions and development of symptoms in the 4 bean varieties: (a) Leaf inoculations, (b) stem inoculations, (c) germinated seed inoculations, and (d) pod inoculations.

Leaf Inoculations. Four or 5 days after inoculation small, water-soaked areas appeared on the under side of leaves of Red Kidney and Bountiful. Within 1 or 2 days typical chlorotic halo areas developed. When heavy inoculations were made and many lesions resulted, part of the inoculated leaf, or in some cases the entire leaf, became chlorotic and wilted. The chlorotic halo lesions usually appeared most prominently from 1 to 2 weeks after inoculation; after this time they gradually became less conspicuous due in part to fading of the green color in the uninfected portion of the leaf.

Following the appearance of primary lesions Red Kidney and Bountiful plants often showed symptoms of systemic infection. These symptoms were first manifested by a slight yellowing and vein-clearing of one or more young leaflets near the growing point. Vein-clearing symptoms ordinarily appeared 8 to 12 days after inoculation and were, in turn, frequently accompanied or followed by stunting, wilting, and premature death of the plants. The development of the symptoms of systemic infection depended to a large extent upon the age and the general vigor of the plant at the time of infec-

[4] Stapp, C. Verfahren zur Prüfung von Bohnen (*Phaseolus vulgaris*) auf Resistenz gegen *Pseudomonas medicaginis* var. *phaseolicola* Burkh., dem Erreger der Fettfleckenkrankheit. Angew. Botanik, **15**: 241–252. 1933.

tion. Inoculation of young, vigorously growing plants gave the highest proportion of systemic infection.

On the varieties Red Mexican and Schwert No. 27 the symptoms developed as small, circular, brownish, inconspicuous necrotic lesions that never enlarged to any noticeable extent. Inoculated leaves did not become yellow or wilted, even though in some cases the number of necrotic lesions was large. No symptoms of systemic infection were ever noted on plants of these 2 varieties, even though infected plants were held until mature. Figure 1 presents a comparison of the leaf symptoms on Schwert No. 27 and the susceptible variety Red Kidney.

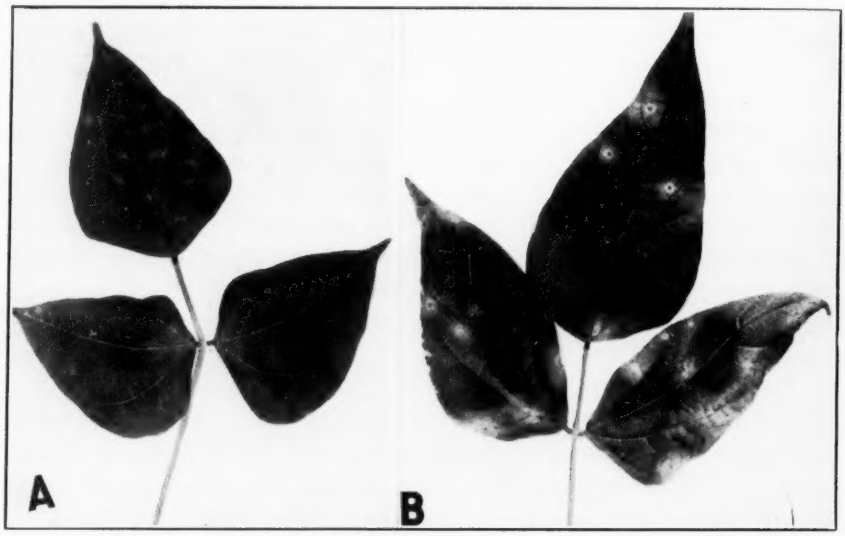

FIG. 1. Symptoms resulting from leaf inoculation with the halo-blight organism. Small, necrotic lesions occur on the leaves of the resistant variety Schwert No. 27 A in contrast with the large halo lesions on the leaves of the susceptible variety Red Kidney B.

In the course of experiments with these 4 varieties, inoculation tests were carried out with plants of all ages from early seedling stage to those nearing maturity. No indications were obtained that the reactions were influenced by age of plant, excepting, of course, that when nearly mature plants were inoculated no leaf symptoms developed.

In a recent paper Goss[5] reported that, under high temperature conditions (28–32° C.), lesions that lack halos appear on leaf-inoculated plants of Red Kidney and U.S. Refugee No. 5, although at lower temperatures (16–20° C.) typical halos were formed on these varieties. In an attempt to determine whether the development of necrotic halo-less lesions on Red Mexican or Schwert No. 27 were influenced by temperature relations, the following experiment was carried out: Forty young plants of each of the 4 varieties were leaf-inoculated. Six hours after inoculation 10 plants of each variety

[5] Goss, R. W. The relation of temperature to common and halo blight of beans. Phytopath. 30: 258–264. 1940.

were placed in the greenhouse in temperature cases held at 16, 22, and 28 degrees C. Within 7 days after inoculation typical halo lesions appeared on leaves of Red Kidney and Bountiful held at usual greenhouse temperatures and in the 22° case, whereas small, necrotic, halo-less lesions developed on Schwert No. 27 and Mexican Red.

At 28° C. lesions without halos appeared 7 days after inoculation on Red Kidney and Bountiful, as reported by Goss for the varieties Red Kidney and U.S. Refugee No. 5. On leaves of Red Mexican and Schwert No. 27 halo-less necrotic lesions appeared about 10 days after inoculation at this temperature.

Since Goss reported that the most prominent halo lesions appeared on susceptible plants held at 12–20° C., it, therefore, seemed probable that if lesions with halos ever occurred on leaves of Red Mexican or Schwert No. 27 they would appear in this range of temperatures. In tests on the effect of temperature only small, necrotic halo-less lesions developed on leaves of Red Mexican or Schwert No. 27 at 16° C. 12 days after inoculation, although large, prominent halo lesions appeared on leaves of Red Kidney and Bountiful plants inoculated at the same time and held in the same temperature-controlled cases. Furthermore, no halo lesions developed on leaves of Red Mexican or Schwert No. 27 although they were held at 16° C. for a period of more than a month after inoculation.

These tests showed that halo symptoms do not appear on inoculated Red Mexican or Schwert No. 27 plants at 28, 22, or 16 degrees C., although halo lesions do occur on Red Kidney and Bountiful at 22 and 16 but not at 28° C. It was concluded from these experiments that necrotic, halo-less lesions developed on leaves of Red Mexican and Schwert No. 27 over a wide range of temperature, and that the reaction was different from the one reported by Goss.

Stem Inoculations. Ten equally old plants of each variety were selected and stem-inoculated when the first trifoliate leaves were about half-expanded. Additional plants to serve as controls were punctured with sterile needles and still other plants were not treated.

Four days after inoculation by the stem-puncture method Red Kidney plants started wilting and began to lose their dark green color. Bountiful plants showed similar symptoms a day or two later. Plants of these 2 varieties that had been punctured with sterile needles remained healthy, as did the untreated controls. Inoculated Red Kidney and Bountiful plants showed vein-clearing, yellowing, wilting, and severe stem cankers with abundant bacterial exudate, and within 3 weeks all were dead.

The behavior of stem-inoculated plants of Red Mexican and Schwert No. 27 was in marked contrast to the above. Although small, watersoaked areas and internal necrosis developed for a short distance around the point of inoculation, the plants continued to grow without the development of any additional symptoms. The punctured controls and untreated plants remained healthy. Figure 2 presents a photograph of plants of the 4 varieties 15 days after they were stem-inoculated.

It was concluded from these tests that systemic infection with halo blight is not induced in Red Mexican or Schwert No. 27 by stem inoculation with strains of the halo-blight bacteria that produced systemic infection in Bountiful and Red Kidney plants. Since these halo strains were the most pathogenic in the collection, it seems highly probable that systemic infection cannot be induced in the two varieties.

Germinated-seed Inoculation. Twenty germinated seeds of each of the 4 varieties were immersed for 2 hours in a bacterial suspension of the halo-blight organism in water. Additional germinated seeds, to serve as controls, were immersed for the same length of time in tap water. After immersion all seeds were planted in soil in clay pots. Within several days water-soaked lesions could be seen on the cotyledons as they unfolded aboveground on all 4 varieties grown from inoculated seed. Later on, lesions appeared

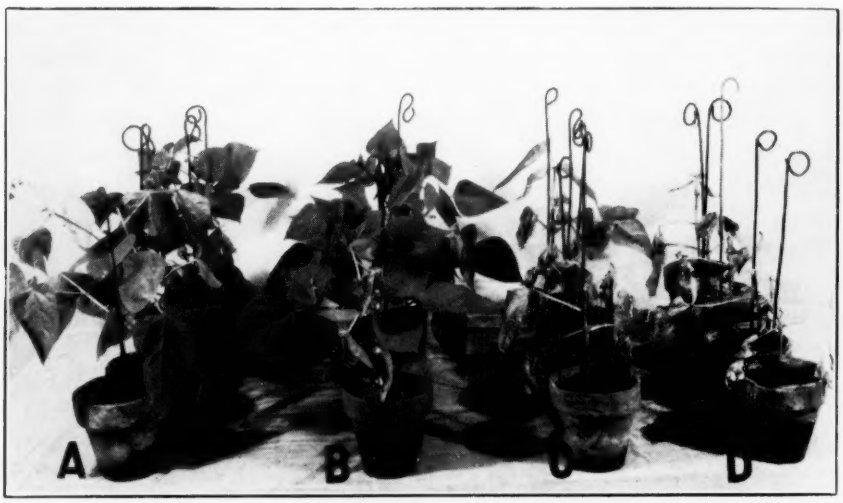

FIG. 2. Bean plants of the four varieties 15 days after stem inoculation with the halo-blight organism. A. Schwert No. 27. B. Red Mexican. C. Red Kidney. D. Bountiful. The two resistant varieties showed no symptoms of systemic infection and produced a normal crop of beans.

on the primary leaves of many plants in all 4 varieties. Within a week nearly all plants of Red Kidney and Bountiful showed symptoms of systemic infection, and, 3 weeks after inoculation, all plants of these 2 varieties were dead. Inoculated Schwert No. 27 and Red Mexican plants, on the other hand, showed no symptoms of systemic infection, but grew to maturity and produced a crop of apparently healthy pods.

These tests further demonstrate a physiological resistance in Red Mexican and Schwert No. 27, as shown by the failure of these varieties to develop systemic infection, even though inoculated and infected in the early seedling stage.

Pod Inoculations. The striking differences that had been obtained in leaf symptoms and in the experiments dealing with systemic infection sug-

gested that some differences might be found in pod lesions on the varieties being studied. Accordingly, 20 plants of each of the 4 varieties were grown in 7-inch clay pots, one plant to each pot, to insure strong, vigorous growth and abundant pod production. When some of the earliest pods of each variety were nearly full-grown, and at a time when each variety was still producing new pods, they were inoculated by the same methods used for leaf inoculations. Within a week after inoculation typical water-soaked lesions appeared on the pods of Red Kidney and Bountiful plants. These enlarged and became typical halo-blight pod lesions. Pods on Schwert No. 27 and Red Mexican, however, developed small, discrete, dry, necrotic spots, which did not enlarge to any extent and, after several weeks, looked like rusty-colored spots caused by mechanical injury. Figure 3 presents photographs of typical pods of the 4 varieties inoculated with halo blight.

FIG. 3. Symptoms resulting from pod inoculations of the four varieties. A. Schwert No. 27. B. Red Mexican. C. Red Kidney. D. Bountiful. The small, discrete, necrotic lesions on the 2 resistant varieties contrast with the larger, water-soaked, coalescing lesions containing bacterial exudate on the pods of the 2 susceptible varieties.

It was concluded from this test that pod lesions on Red Mexican and Schwert No. 27 were markedly different from those produced on susceptible varieties, such as Red Kidney or Bountiful, and that the lack of water-soaking and the small size of the lesions further confirmed the conclusion that these varieties possessed physiological resistance to halo blight.

OTHER RESISTANT VARIETIES[6]

A large number of varieties have been tested either in the field or greenhouse, and, while the detailed results cannot be presented in this paper, it

[6] The variety Princess of Artois was obtained from Canada through the courtesy of T. F. Ritchie. Startler and White Seeded Runner were obtained from Western Australia through the courtesy of W. P. Cass Smith. Drouth Resistant was received from the McFayden Seed Co. of Canada.

should be noted that the following varieties have shown resistance when inoculated by one or more of the methods previously described.

Schwert Nordstern (Stapp 82, F.P.I. 112,771) reacted in a manner similar to the 2 varieties, described in the foregoing as resistant, when inoculated by all 4 methods.

Great Northern, Princess of Artois, Robust, Kaiser Wilhelm (Stapp 100, F.P.I. 112,773) and Marktsieger (Stapp 38, F.P.I. 112,700) were all resistant to germinated-seed and stem inoculations, but gave variable symptoms with leaf and pod inoculations. White Seeded Runner and Zucker-Brech (Stapp 196, F.P.I. 112,775) were resistant to leaf, pod, and stem inoculations, but developed some systemic infection and halo symptoms when germinated seed were inoculated. Startler was resistant with seed, leaf, and stem inoculations, but variable symptoms occurred on the pods; while Blue Lake was resistant to stem and pod inoculations, but gave variable symptoms on the leaves.

In other tests in which leaf and pod inoculations only were used, California Pink and Blue Pod showed resistance and in similar tests no infection was obtained on Drouth Resistant or Arikara Yellow.

BREEDING TRIALS

It seemed possible that crosses between either of the resistant varieties Schwert No. 27 or Red Mexican and desirable commercial varieties, such as Asgrow Stringless Green Pod or Landreth Stringless, would show promise of combining resistance to halo blight with desirable horticultural characters. Accordingly, a number of crosses were made and while sufficient data are not at hand for establishing definite genetical relationships, the preliminary information on such crosses is of interest.

In crosses of either Schwert No. 27 or Red Mexican with Asgrow Stringless Green Pod or Landreth Stringless, all F_1 plants, when inoculated, produced both typical halo lesions and necrotic non-halo lesions. In other crosses with the same parentage, however, segregation occurred in subsequent generations up to the F_5 so that the progeny of certain selections showed necrotic lesions almost exclusively, while those of other selections from the same crosses showed typical halo lesions.

It is, therefore, concluded probable that resistance to halo blight can be combined with desirable horticultural characters, although back-crossing probably will be necessary before desirable resistant varieties of commercial value are produced. Work is being continued along this line.

DISCUSSION

Many bean varieties, reportedly resistant to halo blight in the field, have become severely infected when inoculated in the greenhouse under controlled conditions. Certain other varieties have shown what appears to be a true physiological resistance when subjected to similar tests. Undoubtedly there are several genetic factors involved in the host-parasite relationship with

halo blight. It is likely that some factors exercise control over the entrance of the organism, while others influence the action of toxic substances and the movement and multiplication of the bacteria in the host.

The utilization of the type of resistance here reported in the development of new bean varieties should greatly aid in the control of this disease. Relatively little damage occurs to the yield or the quality of the marketable crop from such infections as have been described. In addition, the small size of the lesions would tend to reduce the production of bacterial exudate and thus limit the amount of inoculum and resultant spread of the disease.

SUMMARY

The development of small, inconspicuous, necrotic lesions on leaves of some bean varieties inoculated with halo blight, instead of the large chlorotic spots that develop on susceptible varieties, suggested that these varieties possess physiological resistance to the disease. These symptoms occurred on leaves of Red Mexican and Schwert No. 27 over a wide range of temperatures, 16, 22, and 28 degrees C., and on plants of all ages from early seedling to plants nearly mature. The inoculation of germinated seed by soaking in a bacterial suspension or of young plants by stem punctures failed to bring about systemic infection. Pod inoculation produced small, rusty-colored, necrotic lesions on these 2 varieties instead of the usual large, water-soaked lesions. It is concluded that these varieties, and others reacting similarly, possess true physiological resistance to halo blight.

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THE EFFICACY OF CERTAIN NEMATOCIDES IN THE CONTROL OF ONION BLOAT IN MUCK SOIL[1]

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(Accepted for publication June 27, 1941)

In a former paper[2] the writers discussed the recent introduction into New York State of the disease of onions (*Allium cepa* L.) known as "bloat," caused by the bulb or stem nematode *Ditylenchus dipsaci* (Kühn) Filipjev. Attempts to eradicate it from the onion fields were described; where steam sterilization was no longer practicable, sulphur and chloropicrin were said to have given some promise as soil nematocides in preliminary field trials on a few farms. Since then, in the early summer of 1940, there has been an increase in the number of onion fields infested with *D. dipsaci*, due apparently to excessive precipitation. The problem has, therefore, become more acute, particularly where growers do not practise a rotation.

It seemed feasible to test the efficacy of certain chemicals as nematocides in onion fields, on the basis of their success in other crops and with other species of nematodes. The use of chloropicrin, ethylene chloride, and mixtures of the two had been reported by Chitwood,[3,4] as applied to sandy-loam soil infested with the daffodil strain of *Ditylenchus dipsaci* and fields infested with the root-knot nematode *Heterodera marioni* (Cornu) Goodey. The results of the first of a series of experiments testing these and other chemicals for the control of onion bloat are given herewith.

PLAN OF EXPERIMENT

One hundred metal cylinders 17 in. long and 2 ft. in diameter, open at both ends, were driven into muck soil to a depth of 12 in. in a field near Watkins, New York. There were 20 experimental treatments (including 3 types of controls) with 5 replications of each, all assigned at random among the 100 cylinders or plots. Three types of inoculum were used and the experiment is outlined, according to inoculum, in the following paragraphs.

(1) In 5 treatments diseased onion bulbs were buried in the soil and served as the source of inoculum. One of these treatments was given no chemical and served as a control, while the 4 remaining treatments received the following chemicals:[5] Chloropicrin at 2cc. per injection to a depth of

[1] We are indebted to Mr. L. B. Reed of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for aid in the statistical analysis.

[2] Newhall, A. G., and B. G. Chitwood. Onion eelworm rot or bloat caused by the stem or bulb nematode, *Ditylenchus dipsaci*. Phytopath. **30**: 390–400. 1940.

[3] Chitwood, B. G. Ethylene chloride for sterilization of soil against nematodes. Florists' Exchange **95**: 9. 1940.

[4] Chitwood, B. G. Soil treatments with volatile liquids for the control of nematodes. Phytopath. **31**: 818–824. 1941.

[5] For method of making treatments see:

Chitwood, B. G. A rapid method for determining *K* values of nematocides. Proc. Helminthol. Soc. Washington **6**: 66–70. 1939; and Frames for spacing injections of soil nematocides. *Ibid.* **6**: 70–73. 1939; and

Taylor, A. L. Efficient spacing of soil fumigants for field applications. *Ibid.* **6**: 62–66. 1939.

6 in., spaced $9 \times 10\frac{1}{2}$ in.; chloropicrin-ethylene chloride (1:9) at 10 cc. per injection to a depth of 6 in., spaced $9 \times 10\frac{1}{2}$ in.; sulphur at $1\frac{1}{4}$ tons per acre mixed with top 6 in. of soil; and sulphur at $2\frac{1}{2}$ tons per acre.

(2) In 14 treatments screened infested soil alone served as inoculum. One of these treatments served as a control; 4 treatments were identical with the chemical treatments given in paragraph (1) and the remaining 9 chemical treatments are listed in table 1.

(3) In one treatment diseased onion tops (leaves) served as the source

TABLE 1.—*Results of various soil treatments on the production of onions and the control of Ditylenchus dipsaci in muck soil (average of 5 replications)*

Treatment	Mean weight of 30 bulbs		Infected bulbs (with bulbs as inoculum)[a]	Efficacy of treatment
	with soil as inoculum	with bulbs as inoculum		
	Grams	*Grams*	*Mean number*	*Per cent*
Control	2909	1298	6.6	0
Chloropicrin (2 cc. $9 \times 10.5''$)	1324[b]	1856	0.2[c]	97[d]
Chloropicrin-ethylene chloride 1:9 (10 cc. $9 \times 10.5''$)	1994[e]	1870	0.0[f]	100[g]
Sulphur $1\frac{1}{4}$ tons per acre	2128	1260	4.8	38
Sulphur $2\frac{1}{2}$ tons per acre	966[b]	708	4.2	31
Chloropicrin (1 cc. $9 \times 10.5''$)	2864	.....	.....	.....
Chloropicrin (3 cc. $9 \times 10.5''$)	692[b]	.....	.....	.....
Chloropicrin-ethylene chloride 1:4 (5 cc. $9 \times 10.5''$)	2986	.....	.....	.....
Ethylene chloride (10 cc. $9 \times 10.5''$)	2904	.....	.....	.....
Crotonaldehyde (10 cc. $9 \times 10.5''$)	3230	.....	.....	.....
Crotonaldehyde (10 cc. $6 \times 7''$)	3018	.....	.....	.....
Mesityl oxide (10 cc. $9 \times 10.5''$)	2814	.....	.....	.....
Mesityl oxide (10 cc. $6 \times 7''$)	3016	.....	.....	.....
Carbon disulphide (10 cc. $9 \times 10.5''$)	2892	.....	.....	.....
Controls (onion tops added)	2528	.....	0.2	.....

[a] No infected bulbs in any case where soil alone was used as inoculum.
[b] Weight reduced odds of 999: 1.
[c] Disease reduced odds of 19: 1.
[d] Efficacy as based on binomial distribution formulae not less than 86 per cent with odds of 19: 1.
[e] Weight reduced, odds of 19: 1.
[f] Disease reduced, odds 99: 1.
[g] Efficacy as based on binomial distribution formulae not less than 91 per cent with odds of 19: 1.

of inoculum. No chemical was applied to this treatment.

At the time the treatments were made on October 5, 1939, the moisture in several samples of the soil ranged from 38 to 52 per cent of the dry weight, the soil temperature varied from 16 to 19° C., while the soil reaction was pH 4.6 to 5.8. Enough water was added to each plot immediately after treatment to facilitate the retention of volatile materials. On April 27 of the following year 5–10–10 fertilizer was applied at the rate of 1200 pounds per acre and 30 healthy onion sets of variety Ebenezer were planted in each plot. Harvest records on mature onions were made on August 6.

EXPERIMENTAL RESULTS

(1) *Control of Ditylenchus dipsaci.* In none of the treated plots or controls where infested but sifted muck was employed could a single onion infected with *D. dipsaci* be found. One infected onion was found in one plot of the control series in which infected onion tops had been buried. The standard error of the difference for comparison of 5 paired treatments (including controls) with or without buried diseased onions equals 0.5161; the minimum mean difference with odds of 999 to 1 is 1.771. The mean number of diseased bulbs per plot where diseased onions were buried was 3.16, and, since none was found in corresponding treatments lacking buried diseased onions, this figure is significant with considerably greater odds than 999 to 1.

Analysis of variance of onion bulbs infected with *Ditylenchus dipsaci* after soil treatment, with onion bulbs as source of inoculum, gave a standard error of the difference for comparison of treatment means of 2.305. The mean difference required for significance with odds of 19 : 1 equals 4.8 and with odds of 99 : 1 equals 6.6. Means of treatments on soil containing buried infected onion bulbs are given in table 1. Two treatments are significant, the 2 cc. chloropicrin and the 10 cc. mixture of chloropicrin-ethylene chloride (1 : 9).

(2) *Other Diseases.* Small percentages of miscellaneous troubles, including fusarium bulb rot, bacterial rot, and onion maggot, were found throughout the plots. Analysis showed that these were not significantly affected by any of the treatments. However, where *Ditylenchus dipsaci* was present in a plot the number of rotten onions was increased. This was apparently due to the entrance of *Ditylenchus dipsaci*-infected bulbs by secondary invaders. It appears probable that this miscellaneous residuum of bulbs infected with some type of disease amounting to means of 0.0 to 2.0 per plot was introduced with the sets.

(3) *Effect on Yield of Bulbs.* The total weight per plot was taken and an analysis of variance conducted. The standard error of the difference for comparison of treatments equals 425.252 grams. The mean differences required for significance at various levels are as follows: with odds of 19 : 1, 848 grams, with odds of 99 : 1, 1125 grams, with odds of 999 : 1, 1459 grams. Comparing the various treatments in which onions served as a source of inoculum, one notes rather larger differences in some instances but these differences are dependent on both treatment and degree of infestation. Comparing the various treatments in which soil served as a source of inoculum, 4 treatments differ significantly from the corresponding control. These treatments are chloropicrin at a dosage rate of 2 and 3 cc. per injection, sulphur at a rate of 2½ tons per acre and chloropicrin-ethylene chloride at a dosage rate of 10 cc. per injection. All of these treatments were detrimental in the present experiment, the chloropicrin-ethylene chloride mixture being the least injurious.

DISCUSSION

Sifted muck soil from a field showing heavy infestation with *Ditylenchus dipsaci* in 1939 did not prove effective as a source of infection in the experiment. It was necessary to add further inoculum in the form of diseased onions or, in one case, leaves from diseased onions. The writers (*loc. cit.*) have reported the failure of previous attempts to obtain infection on seedlings grown in this same sifted soil. The latter half of the summer of 1939 was unusually dry, resulting in warm soil temperatures which may have contributed to the apparent absence of nematodes free in the soil.

Sulphur mixed with the soil in the fall, even at 2½ tons per acre, did not result in a significant reduction of bloat in the next season's crop when diseased onions had been previously buried in the soil.

Chloropicrin, at a dosage rate of 2 cc. in holes $9 \times 10\frac{1}{2}$ in. apart ($390 per acre) reduced bloat significantly. The efficacy was 97 per cent.

Chloropicrin-ethylene chloride mixture (1:9), at a dosage rate of 10 cc. ($294 per acre), eradicated the disease completely.

Sulphur, at 2½ tons per acre and chloropicrin at a dosage rate of 2 and 3 cc. per injection ($9 \times 10\frac{1}{2}$), caused marked reduction in crop weight. Sulphur, at the rate of 1¼ tons per acre, caused some reduction in crop weight (not significant in this experiment). In experiments with 1¼ tons of sulphur per acre, reported elsewhere,[7] the reduction in crop weight was significant, even in the second year. Chloropicrin-ethylene chloride (1:9), at 10 cc., caused some reduction in crop yield, but ethylene chloride at 10 cc. caused no reduction in yield.

CONCLUSIONS

After a dry season infected onions in the soil are the chief source of inoculum of *Ditylenchus dipsaci*. Infected onion tops also may harbor this nematode to a slight extent.

Sulphur, applied to the soil in the fall, was found to be of no significant value as a nematocide in the present experiment. Heavy applications greatly reduced crop weights.

Fall applications of chloropicrin at the rate of 2 cc. in holes $9 \times 10\frac{1}{2}$ in. apart and a mixture of chloropicrin and ethylene chloride (1:9) at the rate of 10 cc. in holes $9 \times 10\frac{1}{2}$ in. apart both gave very highly significant reduction of onion bloat. The latter treatment was better than the former one but the difference was not significant.

Since ethylene chloride alone was used only with sifted soil as inoculum, and since this inoculum was shown to be inadequate, the efficacy of this material by itself in muck soil remains to be proved. It seems highly probable that when used at a rate of 10 cc. in holes $9 \times 10\frac{1}{2}$ in. apart it would give adequate control of *Ditylenchus dipsaci*, as judged by the senior writer's recent results[8] concerning its application in soil infested with the narcissus

[7] Newhall, A. G., and B. G. Chitwood. The Status of Onion Bloat in 1940. U.S.D.A. Bureau of Plant Industry Plant Dis. Rptr. 24: 350–351. 1940.

[8] See footnote 4.

strain of *D. dipsaci*. Ethylene chloride at this rate would cost only $110 per acre. It should be given further trial.

Chloropicrin, either by itself or in mixture, sometimes reduced onion crop weights. It seems possible that it may have been adsorbed on the carboniferous muck soil. Perhaps the metal containers contributed to its retention. Field application in a similar manner produced no crop reduction.

No evidence of crop stimulation was noticeable from any treatment and the only adverse effects on growth were in the sulphur and chloropicrin-treated plots. Field applications of sulphur have also given consistent reductions in crop weights. Some of these have been reported as persisting into the second season after application.[9]

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[9] See footnote 7.

ASCOCHYTA MAJALIS IDENTIFIED ON LILY OF THE VALLEY IN THE UNITED STATES[1]

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The first known record of *Ascochyta majalis*, causing a leaf spot of lily of the valley, in the United States was obtained in August, 1940, when a grower in Bucks County, Pennsylvania, sent specimens to the United States Department of Agriculture. He sought advice concerning this diseased condition of his planting. He stated that the bed, about 50×20 ft. in size, had been maintained for at least 15 years with no trouble previously from plant disease. At this time, however, near the center of the plot an elongated area about 20×2 ft. became entirely discolored, and the green leaves outside this area were more or less generally spotted.

The specimens received in Washington showed striking leaf spots scattered over the blade. They were often elliptical or elongated, reaching 2 cm. in the longer diameter. The spots were brown,[2] sometimes bordered by purple, and surrounding the border was generally a more or less indefinite zone of bright-green (Fig. 1, A and B). Scattered over the surface of the lesions were minute elevations, which proved to be pycnidia (Fig. 1, C). These occupied nearly the entire thickness of the leaf and their color made them visible through the leaf tissue. They were spherical to flattened, bearing 2-celled or occasionally 3- or 4-celled conidia (Fig. 1, D).

The fungus, recognized as a species of *Ascochyta*, proved to be in agreement with *A. majalis*. This is one of the fungi discovered by Massalongo (3) in Verona, Italy, and the description reads as follows:

"*Ascochyta Majalis* sp. nov.—Maculis fulvo-rubiginosis oblongis ambitu lutescentibus; perithecis membranaceis hypodermis amphigenus osculo minute pertusis 140 : 180 μ. in diametro; sporalis hyalinus saepe quadriguttulatis subcylindraceis, raro curvulis utrinque rotundatis, 1-septatis, ad septum interdum subconstrictis 18 : 24 × 4 : 6 μ.

"In foliis subemortuis *Convallariae Majalis*, in cultis vici 'Tregnago': Oct. 1899."

Massalongo again collected this fungus at Tregnago in September, 1901, and this collection was issued in D. Saccardo's Mycotheca Italica as Number 960.

The only other available record of the fungus in Europe is represented by its discovery in the Austrian Alps in July, 1907, by Otto Jaap (4). His record of the fungus, apparently on wild lily of the valley, reads as follows:

"*A. majalis* Mass. Auf lebenden Blättern von *Convallaria majalis* auf der Mendel am Wege zum Penegal.—Blattflecken weichen etwas ab von der Beschreibung; stimmt sonst gut."

[1] Paper presented at the thirty-second annual meeting of The American Phytopathological Society, Philadelphia, Pennsylvania, December 27 to 31, 1940 (2).

[2] On the dry specimen "Hessian brown" according to Ridgway (7).

An actual comparison of the specimen from Pennsylvania with that from Italy collected in 1901 shows that the fungus from the United States is entirely in agreement with that from Europe. In either case the spores tend to be yellowish rather than entirely hyaline. Owing to their sticky walls

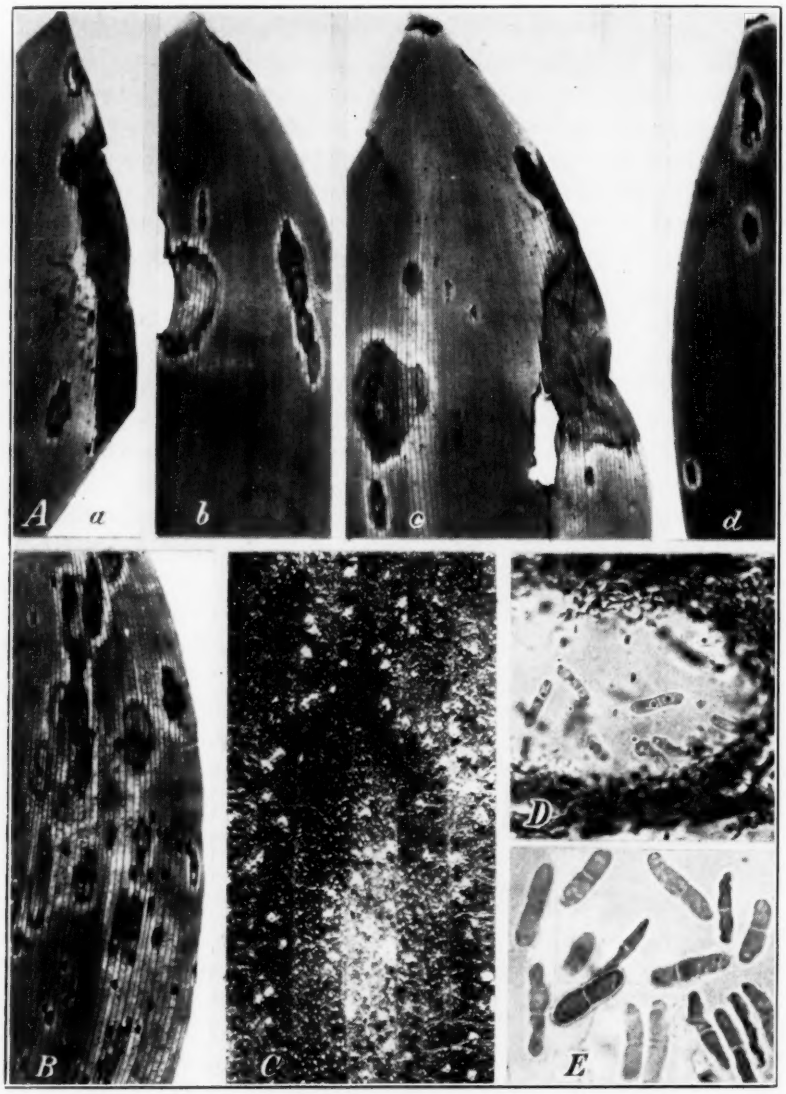

FIG. 1. A, *a–d*. *Ascochyta majalis* on lily of the valley from Bucks Co., Pa., September, 1940. ×1. B. Part of the leaf from the type locality (Italy). ×1. C. Central part of the large leaf spot in A, *c*, showing pycnidia of the *Ascochyta*. ×5. D. Part of a pycnidium, with spores, from the specimen from Pennsylvania. ×300. *E*. Spores from the specimen from the type locality, Verona, Italy, September, 1901 (D. Sacc. Fungi Italica No. 960). ×500. Photographs by M. L. F. Foubert.

they may form agglutinated masses and may not be easily separated from one another. The spores shown in figure 1, E, are from the specimen of 1901 from Italy. It will be noted that a 3-celled spore is represented.

On the material from Pennsylvania the *Ascochyta* was clearly in prominence and there seemed no question that it was responsible for the severely diseased condition in the bed of the lily of the valley concerned. The owner stated that certain younger and more recent beds were apparently healthy; moreover, that in no case had plants from outside sources been introduced into his highly prized and theretofore successful plantings.

The manner in which this little-known fungus reached the bed of lily of the valley is, of course, unknown. But, in its origin in the United States, it would appear that it is an introduction from Europe.

Isolations of the *Ascochyta* from Pennsylvania were made by means of tissue cultures. The plantings were first dipped in a solution of mercuric chloride (1:1000) and then rinsed in sterile water. Potato-dextrose agar medium was employed, and on this substrate a dull white cottony fungus growth soon developed. Later, yellowish, sticky masses of conidia of the *Ascochyta* were present, although pycnidia were not distinguished.

In one case an organism other than the *Ascochyta* was obtained in culture. The planting, which was part of a discolored area on a leaf base, gave no growth for several days, when a pure culture, supposedly of *Kabatiella microsticta* Bubak (1), began to develop. This fungus, already known to the writer and previously reported (5) from the United States, was also formerly discovered in Europe. The type collection, from Bohemia, 1905, by Kabát, is represented by Kabát and Bubák, Fungi Imperfecti Exsiccati No. 435, of which a specimen is available. From Pape's (6) account of a disease outbreak in cultivated lily of the valley in Germany, it seems to the present writer that the *Gleosporium* sp. to which the disease is attributed may prove to be *K. microsticta*.

In autumn, 1940, only this fungus and no *Ascochyta* was found on leaf spots on freshly collected specimens of lily of the valley from two widely separated gardens in Washington, D. C.

BUREAU OF PLANT INDUSTRY,
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LITERATURE CITED

1. BUBÁK, F., and KABÁT, J. E. Mykologische Beiträge IV. Hedwigia **46**: 288–298. 1906–1907.
2. JENKINS, A. E. *Ascochyta majalis* identified on lily of the valley in the United States. Phytopath. **31**: 13. 1941. Abstract.
3. MASSALONGO, C. De nonnullis specibus novis micromycetum agri veronensis. Atti R. Inst. Veneto di Sci., Lett. ed Arti. 59, pt. 2, 1899–1900, p. 684.
4. JAAP, O. Beiträge zur Pilzflora der österreichischen Alpenländer. Ann. Myc. **6**: 192–221. 1908.
5. MARTIN, G. H. Notes on *Kabatiella microsticta* found on lily, iris and lily of the valley. U. S. Dept. Agr. Plant Disease Rptr. **13**: 43–44. 1929.
6. PAPE, H. Gloeosporium-krankheit in Maiblumenkulturen. Gartenw. 31: 344–346. 1927.
7. RIDGWAY, R. Color standards and color nomenclature. 42 p., 52 colored plates. (Washington) 1912.

BACTERIAL WILT OF DENT CORN INBREDS

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Losses from bacterial wilt of dent corn have, until recently, been considered of minor importance. Increasing evidence, however, of the importance of this disease on dent corn developed during the wilt epidemics of sweet corn in 1932 and 1933 and later in 1937 and 1938. During these epidemics, bacterial wilt assumed an unusual, late-season importance on dent corn in the form of a leaf blight. It was reported that late leaf blight in many fields reduced the green leaf area as much as 25 per cent,[2] and that in such fields grain yields were greatly reduced.

On both dent and sweet corn, bacterial wilt begins from the feeding injuries, on the leaves, produced by the corn flea beetle (*Chaetocnema pulicaria*). Although the manner of infection and general symptoms on both kinds of corn are the same, the time and type of injury are different. On sweet corn, early systemic infection is the important phase of the disease. On dent corn, local leaf-blight lesions on maturing plants do the greatest damage.

The young plants of some dent-corn lines show pronounced feeding injury; but, in spite of such injuries, few wilt lesions develop. At this stage in their development the young dent-corn plants appear to be much more resistant to infection than young sweet-corn plants. Records taken at Arlington Experiment Farm, Arlington, Virginia, about the middle of June, have shown that, in 1939, of 15,000 dent-corn inbred plants, 1.5 per cent were infected, as compared with 27 per cent infected sweet-corn plants; and that at the comparable time in 1940, of 12,000 dent-corn plants, 0.7 per cent were infected, as compared with 35 to 46 per cent infected sweet-corn plants. In both seasons the young plants of both dent and sweet corn were in adjacent plots in the same field. On the whole, therefore, young dent-corn plants appear to be much more resistant to natural infection than those of sweet corn.

A change in the resistance of dent-corn plants to bacterial wilt apparently takes place in late July and August, after the plants have tasselled. At this season, in Virginia and Maryland, as many as 80 per cent or more of the corn flea beetles are carrying the wilt organism and can transmit the bacteria to the dent-corn leaves. Local, light-green to yellow spots and streaks begin to appear on the lower leaves. Through August and September, these leaf spots may increase rapidly in size and number on susceptible lines, and spread from the lower to the upper leaves. Infected leaf tissue soon dies and becomes dry. Heavily infected leaves, whipped by the wind,

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[2] Koehler, Benjamin. Several corn diseases unusually prevalent in Illinois. Pl. Dis. Rptr. 22(18): 374. 1938.

become torn and ragged. In heavily infected varieties, the light-colored dead tissue gives a row or field the appearance of having been frosted. Very susceptible varieties may become so heavily infected that whole plants become prematurely dry.

It is this late leaf blight in July, August, and September that is the important phase of bacterial wilt on dent corn. In susceptible varieties, losses of green leaf area have been estimated as large as 50 per cent, and such infection apparently increases susceptibility to diplodia stalk rot.[3]

With the increasing acreage of hybrid corn, it is important to know the reaction to bacterial wilt of inbred parent lines, as well as of the hybrids. Preliminary tests of the reaction of inbred lines of dent corn to bacterial wilt were begun at Arlington Experiment Farm in 1939 and continued in 1940. In order to test their relative resistance in early, as well as late, stages of growth, records were kept of the amount of infection resulting from field inoculations of young plants and of the amount of leaf blight late in the season on the same maturing inbreds. Ten plants each of 800 to 900 inbred lines were inoculated when 10 to 12 inches tall to determine differences in susceptibility in early stages of growth. The results were recorded in ratings of 1 to 5, 1 being resistant; 2, moderately resistant; 3, moderately susceptible; 4, susceptible, and 5, very susceptible. Later in the season leaf blight developed naturally on noninoculated plants in the same rows, and differences were recorded by the same ratings, 1 to 5. The results of these ratings are given in table 1.

Inoculations of the young plants were made by hypodermic injections into the stalk about 1 inch above the ground. The inoculum was a water suspension of 3 virulent cultures of *Xanthomonas stewarti* (E. F. Smith) Dowson. In the 1939 inoculations the fibrovascular bundles were wounded somewhat more than in 1940. The results of inoculations for 1939 and 1940 are given in the 2 left-hand columns and the leaf-blight ratings in the right-hand columns. The inbreds are in the order of their relative resistance to the artificial inoculation of the young plants. While the results are not identical for the 2 years they appear to be reasonably uniform.

As shown in table 1, the inbreds vary in susceptibility to inoculation in the seedling stage from resistant (1) to very susceptible (5). At least 12 lines were very resistant in both seasons. Eight were moderately susceptible (3) and KYS was very susceptible (5). The plants of KYS, when inoculated, made no further growth and wilted and died.

The results of the inoculations show greater variability in 1940 and, in most instances, less infection than in 1939. This lighter infection is probably due to the less vigorous inoculation methods used in 1940.

From the leaf-blight records in the right-hand columns, with one exception, these inbreds are all more susceptible to leaf blight as the plants are maturing than to inoculation in early stages of growth. Inbred Ia. L317

[3] Holbert, J. R., Charlotte Elliott, and Benjamin Koehler. Bacterial leaf blight of dent corn. Phytopath. 23: 15–16. 1933.

TABLE 1.—*Bacterial wilt ratings of a selected group of inbred lines of dent corn grown at Arlington Experiment Farm, Arlington, Virginia, 1939 and 1940*

Inbred lines	Wilt ratings[a] on			
	Young plants hypodermically inoculated		Mature plants naturally infected with leaf blight	
	1939	1940	1939	1940
Ky. 27	1	1	2	1–2
US 23	–	1	2	2
Mo. G	1	1	2	2
Ky. 39	–	1	2	2
US 24	–	1	3	2
US 21	1	1	3	2
Ill. Hy	1	1	3	2
Ind. 38–11	1	–	3	3
Ind. P8	1	1	3	3
US 41	1	1	3	3
K58	1	–	4	2
Ky. 30A	1	1	4	2
Ia. I205	1	1	4	2
Ohio 51	1	1	4	2
N. J. B42	1	1	4[b]	2–3
Ia. I234	1	–	4	3–4
Ia. B1 345	1	–	4	4
Ia. B1 349	1	–	4	4
Ill. A	1	1	4[b]	4
Ill. 4226	1	1	4	4
T. 18C	1	1–2	3	1
T. 10B	1	1–2	3	2
Ia. KB 397	1	1–3	4	3–4
US 3–(S6)–4–1	1	1–4	2	3
Ill. R4	1	2–3	3	3
Ohio 67	1	2	3	2
Ind. Tr.	1–2	–	4	3–4
US 6–(S6)–2	1–2	1–2	3	4
Ind. WF 9	2	1	3	2–3
US 5–(S6)–1–1	2	1	4[b]	4
Ind. B2	2	1	4[b]	2
Ia. I197	2	1	4	3
US 43	2	1–2	4	3
US 62	2	1–2	3	3
Ia. WD 456	2	1–2	4	3
Tx. 158–4	2	1–2	3	2–3
Ohio 56	2	2–3	4	4
Ia. Os420	2–4	1–4	4	3–4
US 7–(S5)–1–1	3	1	3	3
N. J. A64	3	1	4	4
Ill. 90	3	1–2	3	2
US 33(S10)–3	3	1–2	3	2–3
N. J. A12	3	1–2	4	2–3
US 61	3	1–3	3	2–3
Ia. L289	3	1–3	3	3
Ia. L317	3	1–4	2	1
US 2–(S6)–1	3	1–4	3	4
Ia. ITE 701	3	2	4	4
Tx. 102A	3	2	–	3
KYS	5	1–4	5	3

[a] Grade 1 = resistant, and grade 5 = very susceptible.

[b] In some inbreds the record is doubtful. Plants became prematurely dry.

was somewhat more susceptible to seedling inoculation than to late natural infection as leaf blight. Seedling reaction to inoculation apparently is not a safe indication of susceptibility to leaf blight in maturing plants. This is borne out under natural conditions in the field where leaf blight is not only much more abundant but also more destructive than early, primary infection. Leaf-blight ratings are fairly uniform for each strain for the two years, but, on the whole, there was somewhat less leaf blight in 1940 than in 1939.

These differences in the resistance of dent-corn inbreds to bacterial leaf blight indicate promising possibilities for the development of corresponding resistance in dent-corn hybrids. This is of special importance as the use of resistant strains is the only practical means of controlling bacterial leaf blight.

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CONSERVATION OF SCHOLARLY JOURNALS

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.